THE INHERITANCE OF ERUCIC, EICOSENOIC AND OTHER FATTY ACID COMPONENTS OF RAPESEED OIL (*BRASSICA NAPUS L.*)

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ABSTRACT

THE INHERITANCE OF ERUCIC, EICOSENOIC AND OTHER FATTY ACID COMPONENTS OF RAPESEED OIL (*BRASSICA NAPUS L.*)

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The fatty acid compositions of oil from cross-pollinated seed from four parental strains indicated that both the genotype of the maternal sporophyte and the genotype of the developing seed influence the composition of the seed oil. Oleic, linoleic and linolenic acid contents are influenced by the maternal sporophyte. Eicosenoic and erucic acid contents of the seed oil are determined by the genotype of the developing seed and not by the genotype of the maternal sporophyte.

Genetic analysis of backcross populations derived from three parental strains distinctly supports the hypothesis that erucic acid content of seed oil is determined by a two gene system. These analyses also indicated that eicosenoic acid content of seed oil is determined by a two gene system. The regular association of eicosenoic and erucic acid values indicates that the same genes control the content of both fatty acids. The same gene system, which acts in an additive manner with respect to erucic acid, acts

in a dominant manner for eicosenoic acid. The inheritance of oleic and linoleic acid appears to be complex.

INTRODUCTION

According to Downey and Bolton (7), rapeseed as an oilseed crop was first grown commercially in Canada in 1942, when Black Argentine rape (*Brassica napus L.*) was imported from Argentina and distributed to farmers. The growing of rape in Canada was a war measure to supply rapeseed oil for use as a marine engine lubricant. Price support of 6¢ per pound was maintained during the period from 1942 to 1948, during which time the acreage rose to eighty thousand acres. The number of acres declined rapidly with the discontinuation of price supports.

Bell, Downey and Wetter (1) stated that Canadian rapeseed is a major item in the edible-oil markets of the world. Canada's exports have averaged 148,000 tons per year since 1957, which is more than the total exports of rapeseed from all other countries. Home consumption of rapeseed oil and meal is increasing, but the markets of Europe, Africa and Asia still continue to be the major outlets.

In the current Canadian varieties the average oil content (dry basis) is 42.4%, ranging from 38% to 44%. Since a new high in average yield of rapeseed in Canada has been reached, the researcher has placed more emphasis on the investigation of the quality of rapeseed oil. It was indicated by

Downey (6) that investigation of variation in oil composition, that is quality, is very important since the acceptability of a vegetable oil for any specific purpose is largely determined by its content of fatty acids. For industrial purposes oils high in linolenic, linoleic, or erucic acid are valued, but edible oils high in oleic and linoleic and low in linolenic are highly favoured. The latter group is exemplified by safflower, sunflower, corn and peanut oils.

Rapeseed oils differ from the major edible oils in that they have a high percentage of erucic acid (25--40%). To make rapeseed oil competitive with other premium edible oils it would appear desirable to reduce erucic acid to zero per cent. The low percentage of palmitic acid present in rapeseed oil is desirable in salad oils.

In the genus Brassica erucic acid content was the first to be studied because of the ease of measurement. At one time erucic acid was considered undesirable for edible oil purposes. Its importance in determining whether an oil was useful for industrial or edible purposes was another major factor.

The fact that the fatty acid composition of the seed is largely controlled by the genetic constitution of the embryo (8), but is dependent upon the raw materials supplied by the parent plant indicates complicating environmental factors in determining the mode of inheritance. The issue is further complicated by the fact the *B. napus* is an amphidiploid (13).

This study was undertaken to provide some information on the genetic basis of the inheritance of the fatty acid composition of seed oil in *B. napus*.

LITERATURE REVIEW

Craig and Murty (4) investigated the use of gas-liquid-phase chromatography (G.L.P.C.) for semimicro- and micro-analysis of mixtures of saturated and unsaturated fatty acids. The potential usefulness of G.L.P.C. as an analytical tool in plant breeding was emphasized by Craig (2).

Craig and Wetter (5) reported on varietal differences in fatty acid composition of rapeseed oil in *B. napus* and *B. campestris*. The authors noted a wide range of erucic acid content, 22.4% to 49.2%. This was confirmed by another report by Craig (3) in a study of fatty acid composition of 1958 varietal tests.

Stefansson and Hougen (11) reported a wide variation in erucic acid content of seed oil within varieties of the species *B. napus*. The wide variation within the variety Liho (*B. napus*), a German forage variety, was particularly interesting. Some plants with less than ten per cent erucic acid content in seed oil were isolated and it was noted that oleic acid replaced the erucic acid. Investigation into the inheritance of erucic acid content was initiated immediately after Stefansson, Hougen and Downey (12) reported the isolation of rape plants, from the Liho variety, having seed oil with zero per cent erucic acid. Later, plants of another strain, Budapest (*B. napus*), were isolated with zero per cent erucic acid.

Published studies on inheritance of fatty acid composition in the species B. napus are very few. This is also true for other oilseed crops. Harvey (8) carried out a study on the inheritance of erucic acid content in B. napus. The results indicate a two gene system operating in an additive manner.

Craig (3) in his investigation of 1958 varietal tests showed a significant negative correlation between oleic and erucic acid content. Due to the large number of stations, that is wide range in geographical latitude, analysis of variance showed significant differences between stations for all fatty acids. This indicates a marked environmental effect which would tend to complicate any attempt at genetic analysis.

The high degree of environmental effect was also demonstrated by Siemens (10) in showing the relationship of the iodine value of the mature seed to different temperature treatments at different periods during flowering and maturation of the seed. Under constant temperature treatments it was shown that there was a pronounced negative association between iodine number and temperature. For plants grown at 60°, 70°, and 80° Farenheit, the iodine values were 99.7, 97.9, and 87.9 respectively. These values were significant at $P = 0.05$. It was also demonstrated that the temperature during the period after flowering was most effective in determining the iodine value.

Some information on inheritance of fatty acids is available in other unrelated oil crops. White, Quackenbush and Probst (14) studied the occurrence and inheritance of linolenic and linoleic acids in soybean seeds. Their results indicated that inheritance was quantitative and they noted transgressive segregation to low amounts of linolenic acid in F_2. They demonstrated a significant correlation between linoleic and oleic acids. Knowles and Mutwakil (9) investigated the inheritance of iodine values in safflower. Their results indicate a simple one gene system. The following designations were given to each genotype: OlOl, Olol, and olol having iodine values 131--145, 111--130 and 75--90, respectively. The group 131--145 has oleic 16%, linoleic 76%; the intermediate group 111--130 has oleic 36%, linoleic 57%; and the low group 75--90 has oleic 87% and linoleic 8%. In this case the fatty acid composition of the seed was controlled by the genotype of the embryo, as indicated in rapeseed (Harvey (8)). Yermanos and Knowles (15) reported on the fatty acid composition of cross-pollinated seed of flax. They noted that where two parents differ in the proportion of major fatty acids in their seed, self- and cross-pollinated seeds developing on the same plant do not have the same relative fatty acid composition. This indicates that the fatty acid composition of the seed is controlled by the genotype of the embryo. However, the composition of the cross-pollinated seed of reciprocal crosses was not equal but the fatty acid composition tended to approach the value of the maternal parent, that is, a definite effect of the maternal sporophyte on the fatty acid composition of the seed.

Lack of information on the inheritance of fatty acid composition in oilseed crops indicates the necessity of more genetic investigation.

MATERIALS AND METHODS

The four strains used in this genetic study were isolated from strains used in the rapeseed breeding program at the University of Manitoba. All four strains belong to the species Brassica napus. The strains were chosen because of the contrasting fatty acid composition of their seed oils. "Zero erucic" strains came from both Liho and Budapest strains. Two strains were isolated from Liho, a "zero erucic", and a "zero erucic, high linoleic". A "zero erucic" strain was isolated from Budapest and a "high erucic" strain from Nugget. The "zero erucic" strains actually contain 0.2 to 0.8% erucic acid in the seed oil but are classified as zero per cent for this study. The fatty acid compositions of the strains are presented in Table I.

Seed of the parental strains was planted in the field in May 1962. Diallel crosses and their reciprocals were produced using the four parental strains. Plants in the field were destroyed by hail. The parental strains were reseeded in the greenhouse July 1962. Diallel crosses, their reciprocals and self-pollinated seed were produced on each parent plant. The cross-pollinated and self-pollinated seed was harvested in January 1963. Self-pollinated seed of each parent was analyzed to make certain that the parental plants were representative of each strain.

TABLE I

DESCRIPTION OF THE PARENTAL STRAINS AND THE FATTY ACID COMPOSITION OF THEIR SEED OIL

Description of strain	Fatty acids as per cent of total fatty acids					
	Palmitic	Oleic	Linoleic	Linolenic	Eicosenoic	Erucic
"Zero erucic" from Liho	5.0	62.0	23.5	8.0	1.5	trace
"Zero erucic, high oleic" from Budapest	4.0	74.0	13.0	8.0	1.0	trace
"Zero erucic, high linoleic" from Liho	6.0	46.0	36.0	10.5	1.5	trace
"High erucic" from Nugget	4.0	22.0	15.0	7.0	14.0	38.0

Self- and cross-pollinated seed of the selected parents was planted in the greenhouse in January 1963. The F_1 plants were backcrossed to both parental strains using the F_1 as the male parent and reciprocal backcrosses were also made. Parental strains and F_1 plants were self-pollinated. The seed was harvested from the greenhouse in May 1963 and was planted in the field to produce progeny rows from parental strains, F_1 backcross populations and F_2 populations.

Fatty acid composition of seed oils from self-pollinated seed of parents harvested in the greenhouse January 1964 and May 1964 indicated that there was little variation within a strain. However, substantial differences in oil composition occurred between winter and spring harvested seed. The comparisons are listed in Table II.

Precautions were taken to minimize the environmental effects on fatty acid composition. To make the fatty acid compositions comparable, self-pollinated seed from original parents and self-pollinated seed of backcross parents were planted in the field at the same time with the backcross and F_2 populations.

The following were self-pollinated in the field: progeny of original parents, progeny of backcross parent, backcross populations, and F_2 populations. In each backcross population an attempt was made to obtain self-pollinated seed on one hundred plants. Backcross populations were used in preference to F_2 populations because the backcross

TABLE II

COMPARISON OF FATTY ACID COMPOSITIONS OF SEED OIL FROM TWO DIFFERENT HARVEST DATES

Strain	Date of harvest	Fatty acids as per cent of total fatty acids					
		Pal-mitic	Oleic	Lino leic	Lino-lenic	Eico-senoic	Eru-cic
"Zero erucic" from Liho	Jan.1963	5.3	68.1	20.6	5.7	0.3	trace
	May 1963	4.6	59.8	24.5	8.4	1.5	trace
"Zero erucic, high oleic" from Budapest	Jan.1963	5.2	74.8	11.6	6.2	2.2	trace
	May 1963	4.5	68.8	15.7	9.3	1.6	trace
"Zero erucic, high lino-leic" from Liho	Jan.1963	7.0	41.3	37.6	12.7	1.4	trace
	May 1963	5.7	48.0	30.8	12.7	2.1	trace
"High erucic" from Nugget	Jan.1963	4.1	32.1	14.2	5.0	12.5	32.1
	May 1963	3.9	22.5	15.7	7.5	14.5	35.9

populations are more efficient for genetic analysis. The efficiency is due to the fact that a smaller number of genetic classes is expected and therefore smaller populations required.

Self-pollinated seed was produced by taking one of the upper branches and removing all opened florets. All but eight to ten of the largest unopened florets were removed from the tip of the inflorescence. The group of florets was then tagged and covered by a 3x8" pollinating bag (no. 317 "Central States Pollinating Bags") which was firmly clipped around the main branch of the plant. The pollinating bag remained on the inflorescence until four or five days after all florets had opened and shed pollen. The bag was then removed to prevent damage to the inflorescence in the bag due to rapid elongation of the inflorescence and to discourage aphid infestation. This method was generally successful in producing self-pollinated seed. In some cases self-pollinated seed was difficult to obtain, but this seemed to be a characteristic of specific populations.

Bulk samples of self-pollinated seed from each individual plant were used for analysis rather than the single seed method (Harvey (8)), because bulk samples were expected to minimize environmental effects. Bulk samples consisted of over twenty seeds; most samples were fifty seeds or more.

A Carver press was used for extraction of oil. The seeds were placed in a Carver test cylinder and pressed at a pressure of 1150 psi. for a period of fifteen seconds. The seed and oil were washed with Skellysolve F (petroleum ether) from the cylinder into a vial and were allowed to stand for at least one hour. The oil and Skellysolve F solution were decanted into 30ml. flasks with bottoms drawn to a point. To produce methyl esters of the free fatty acids, five ml. 0.02N sodium methanoate ($NaOCH_3$) was added, followed by refluxing for ten minutes at 50--60°C. After the refluxing, the methyl ester solution was neutralized by adding five ml. 0.02N acetic acid. The solution was washed with 30 ml. distilled water and finally the Skellysolve F was evaporated from solution leaving the methyl esters of the fatty acids.

Gas-liquid-phase chromatography (G.L.P.C.) was used to determine the fatty acid composition of seed oil. The G.L.P.C. unit used in this study was a Beckman GC-2A equipped with a Philips strip chart recorder. Areas of the peaks were determined by the use of a Siemans integrator incorporated into the recorder.

The major fatty acids in rapeseed oil are: palmitic, (16 carbon chain, saturated), oleic (18 carbon chain, one double bond), linoleic (18 carbon chain, two double bonds), linolenic (18 carbon chain, three double bonds), eicosenoic (20 carbon chain, one double bond) and erucic (22 carbon chain, one double bond). Several minor fatty acids occur in small quantities in rapeseed oil (palmitoleic, eicosadienoic, behenic and arachidic, each less than 1%). Analysis of these minor constituents was sacrificed to save time and to permit rapid analysis of the large number of samples required for this genetic study.

Column specifications were as follows:

1. Tube: copper, 36"x3/16" plus adapter to 1/4", Beckman GC-2A
2. Solid phase: 40/50 mesh firebrick
3. Liquid phase: Diethyleneglycolsuccinate
4. Temperature of column: 193°C.
5. Carrier gas: helium

Since several columns were used during the investigation, the pressure was varied to give a total time for each sample of approximately twenty minutes from injection to the end of erucic acid peak.

RESULTS AND DISCUSSION

I. EFFECT OF POLLEN SOURCE AND MATERNAL PARENT ON OIL COMPOSITION

The effect of pollen source and maternal parent on oil composition was studied in diallel crosses involving the four parental strains. Cross- and self-pollinated seed from several pairs of parents was analyzed for fatty acid composition (Table III). Since the initial seed produced in the field was damaged by hail, the seed used for these analyses was obtained under greenhouse conditions and harvested in January 1963.

The erucic acid contents of oil from reciprocally cross-pollinated seeds between "zero" and "high" parents were essentially equal (Table III). The erucic acid content of the oil from cross-pollinated seed was higher than the average of the erucic acid content of oil from self-pollinated seed from the two parents. According to published results, the erucic acid value of cross-pollinated seed should be equal to the average of the two parents (Harvey (8)). Under greenhouse conditions the maximum erucic acid values for the "high" parents were 30% whereas the maximum values under field conditions were 41%. The failure of the "high" parent to express its full genetic potential under greenhouse conditions gives a possible explanation for the fact

TABLE III

FATTY ACID COMPOSITION OF SELF-POLLINATED AND CROSS-POLLINATED SEED FROM THE FOUR PARENTAL STRAINS

Cross	Fatty acid composition as per cent of total fatty acids					
	Palmitic	Oleic	Linoleic	Linolenic	Eicosenoic	Erucic
"Zero erucic" from Liho x "zero erucic" from Budapest						
"zero erucic" from Liho (self-pollinated seed)	5.3	66.6	19.4	7.3	1.4	--*
cross-pollinated seed	5.9	64.4	18.6	8.5	2.5	--
cross-pollinated seed (reciprocal)	4.9	75.5	13.1	6.8	2.7	--
"zero erucic from Budapest (self-pollinated seed)	5.2	74.8	11.6	6.2	2.2	--
"zero erucic" from Liho (self-pollinated seed)	4.6	65.4	22.1	7.2	0.7	--
cross-pollinated seed	5.8	62.6	21.3	8.4	1.9	--
cross-pollinated seed (reciprocal)	2.4	76.1	13.8	6.2	1.4	--
"zero erucic" from Budapest (self-pollinated seed)	5.2	74.8	11.6	6.2	2.2	--

* Denotes trace quantity

TABLE III (continued)

Cross	Fatty acid composition as per cent of total fatty acids					
	Palmitic	Oleic	Linoleic	Linolenic	Eicosenoic	Erucic
"Zero erucic" from Liho x "zero erucic, high linoleic" from Liho						
"zero erucic" from Liho (self-pollinated seed)	5.8	66.8	19.8	5.8	1.8	--
cross-pollinated seed	4.9	63.1	23.8	6.6	1.6	--
cross-pollinated seed (reciprocal)	7.1	41.9	38.5	11.8	0.7	--
"zero erucic, high linoleic" from Liho (self-pollinated seed)	6.3	50.8	32.6	9.4	0.9	--
"zero erucic" from Liho (self-pollinated seed)	5.8	66.8	19.8	5.8	1.8	--
cross-pollinated seed	6.2	54.0	28.1	9.5	2.2	--
cross-pollinated seed (reciprocal)	5.8	46.1	31.8	13.6	2.7	--
"zero erucic, high linoleic" from Liho (self-pollinated seed)	5.7	54.2	30.5	8.7	0.9	--

TABLE III (continued)

Cross	Fatty acid composition as per cent of total fatty acids					
	Pal-mitic	Oleic	Lino-leic	Lino-lenic	Eico-senoic	Eru-cic
"Zero erucic" from Liho x "high erucic" from Nugget						
"zero erucic" from Liho (self-pollinated seed)	5.1	64.0	21.9	7.7	1.3	--
cross-pollinated seed	4.4	33.2	20.4	7.1	12.8	22.1
cross-pollinated seed (reciprocal)	3.3	45.0	11.5	3.5	16.0	20.8
"high erucic" from Nugget (self-pollinated seed)	3.7	41.1	11.7	3.9	14.0	25.6
"zero erucic" from Liho (self-pollinated seed)	5.5	67.4	19.5	6.0	1.5	--
cross-pollinated seed	4.9	31.0	21.5	7.4	12.3	23.0
cross-pollinated seed (reciprocal)	3.4	44.1	11.9	4.2	15.9	20.5
"high erucic" from Nugget (self-pollinated seed)	3.7	41.1	11.7	3.9	14.0	25.6

TABLE III (continued)

Cross	Fatty acid composition as per cent of total fatty acids					
	Pal-mitic	Oleic	Lino-leic	Lino-lenic	Eico-senoic	Eru-cic
"Zero erucic" from Budapest x "zero erucic, high linoleic" from Liho						
"zero erucic from Budapest (self-pollinated seed)	5.3	78.0	9.6	5.3	1.8	--
cross-pollinated seed	4.5	71.6	15.6	6.6	1.7	--
cross-pollinated seed (reciprocal)	5.4	58.2	24.8	10.4	1.3	--
"zero erucic, high linoleic" from Liho (self-pollinated seed)	5.5	57.4	28.1	8.3	0.7	--
"zero erucic" from Budapest (self-pollinated seed)	5.3	78.0	9.6	5.3	1.8	--
cross-pollinated seed	4.4	57.3	24.0	12.0	2.4	--
cross-pollinated seed (reciprocal)	6.9	48.3	31.0	12.2	1.7	--
"zero erucic, high linoleic" from Liho (self-pollinated seed)	5.8	52.5	31.1	9.5	1.1	--

TABLE III (continued)

Cross	Fatty acid composition as per cent of total fatty acids					
	Pal-mitic	Oleic	Lino-leic	Lino-lenic	Eico-senoic	Eru-cic
"Zero erucic, high linoleic" from Liho x "high erucic" from Nugget						
"zero erucic, high linoleic" from Liho (self-pollinated seed)	5.4	53.7	31.8	7.9	1.2	--
cross-pollinated seed	5.0	21.9	24.8	10.0	11.3	26.9
cross-pollinated seed (reciprocal)	4.1	35.8	15.7	4.3	14.7	25.4
"high erucic" from Nugget (self-pollinated seed)	4.0	36.2	12.5	4.3	14.5	28.5
"zero erucic, high linoleic" from Liho (self-pollinated seed)	7.0	54.3	29.4	7.9	1.4	--
cross-pollinated seed	4.9	24.5	22.5	9.0	13.5	25.7
cross-pollinated seed (reciprocal)	3.8	32.5	18.7	6.2	14.2	24.6
"high erucic" from Nugget (self-pollinated seed)	4.3	32.6	15.2	5.4	13.9	28.6

TABLE III (continued)

Cross	Fatty acid composition as per cent of total fatty acids					
	Palmitic	Oleic	Linoleic	Linolenic	Eicosenoic	Erucic
"Zero erucic" from Budapest x "high erucic" from Nugget						
"zero erucic" from Budapest (self-pollinated seed)	5.1	83.0	7.1	4.0	0.8	--
cross-pollinated seed	3.5	45.0	10.0	5.5	17.5	18.5
cross-pollinated seed (reciprocal)	4.3	44.4	10.7	5.4	18.2	17.1
"high erucic" from Nugget (self-pollinated seed)	3.4	34.2	11.2	4.1	17.4	29.7

that the oil from the cross-pollinated seed contained erucic acid exceeding the average of the two parental values. The results which indicate that erucic acid content of the seed oil is determined by the genotype of the developing seed rather than by the genotype of the maternal plant are in agreement with published results (Harvey (8)).

Only "zero erucic" values were obtained from the cross-pollinated seed produced from crosses between "zero erucic" parents derived from the same and different sources. Lack of transgressive segregation suggests that the genes controlling erucic acid content of seed oil in Liho and Budapest strains are the same genes or occupy the same loci.

The eicosenoic acid contents of oils from reciprocally cross-pollinated seed between "low eicosenoic" and "high eicosenoic" parents were not quite equal (Table III). In crosses involving a "high eicosenoic" maternal parent the erucic acid content of the oil from cross-pollinated seed was equal to the erucic acid content of the oil from the self-pollinated seed of the maternal parent. Where the maternal parent was of the "low eicosenoic" type the eicosenoic acid values of the cross-pollinated seeds were somewhat less than the values for the "high eicosenoic" parent but approached the high value. These results indicate that the genes for "high eicosenoic" acid values are dominant and the maternal sporophyte may exert some effect on the eicosenoic acid values of seed oil.

The data on oleic acid content of seed oil from reciprocally cross- and self-pollinated seed appears to be complicated and difficult to explain (Table III, page 16). However, there are known interrelationships between oleic and erucic as well as oleic and linoleic acid contents of the seed oil. For this reason it is necessary to consider the oleic acid data in groups related to their fatty acid components. In each case the oleic acid value of oil from reciprocally cross-pollinated seed from the cross between "zero erucic" from Liho and "zero erucic" from Budapest was equal to the maternal parent (Table III, page 16). Thus in this case the oleic acid content of seed oil is determined by the maternal sporophyte.

The results from the cross-pollinated seed of the cross between "zero erucic" from Liho and "high erucic" from Nugget were different because differences in erucic acid content were involved. The oils from reciprocally cross-pollinated seed were not equal in oleic acid content. Where the maternal parent was of the "high oleic, zero erucic" type the oleic acid values of cross-pollinated seed were lower than both parents but the eicosenoic and erucic acid values were higher. With the "low oleic, high erucic" maternal parent, the oleic acid values of cross-pollinated seed were higher than the maternal parent but the erucic acid values were lower.

The oleic acid values of reciprocally cross-pollinated seed from the cross between "zero erucic" from Budapest and "high erucic" from Nugget were equal. The cross involving the "high oleic, zero erucic" maternal parent produced cross-pollinated seed with oleic acid values lower than the oleic acid values of the self-pollinated seed from the maternal parent, but the eicosenoic and erucic acid values were higher. Where the maternal parent was of the "low oleic, high erucic" type the cross-pollinated seed had a higher oleic acid value than the maternal parent, but the eicosenoic acid value was lower.

In some of these crosses the maternal parent appeared to determine oleic acid content of oil of the cross-pollinated seed, and in other crosses no maternal effect was apparent. The maternal effect was observed in the "zero erucic" crosses where the differences between the parents involved oleic, linoleic and/or linolenic acid values. The maternal effect was not observed in crosses where the major difference involved oleic, eicosenoic and erucic acid values.

The statements in the preceeding paragraph apply to all crosses except those involving one parent, "zero erucic, high linoleic" from Liho. The results from all crosses involving this parent were somewhat inconsistent. In this case, there appears to be an interaction between genotype of the maternal sporophyte and the genotype of the developing seed. The variability of this interaction indicates that the "high linoleic" parent may not have been completely homozygous for genes conditioning oleic and linoleic acid content of seed oil. For this reason crosses involving this parent will not be discussed further.

The linoleic acid contents of oil from reciprocally cross-pollinated seed in crosses between "low" and "high linoleic" parents were not equal (Table III, page 16). The linoleic acid values of the cross-pollinated seed were equal to maternal parent value. In all crosses except those involving the "zero erucic, high linoleic" from Liho, the maternal sporophyte appeared to determine the linoleic acid content of the seed oil.

The linolenic acid values of the reciprocally cross-pollinated seed between "low" and "high" linolenic parents were not equal (Table III, page 16). In the crosses involving a "low linolenic" maternal parent the linolenic acid value of the cross-pollinated seed was equal to the maternal parent value. Where the maternal parent was of the "high linolenic" type the linolenic acid value of the cross-pollinated was equal to or exceeded the maternal parent value. In all cases except those involving "zero erucic, high linoleic" from Liho, the maternal sporophyte appeared to determine the linolenic acid content of the seed oil.

II. ERUCIC ACID

The inheritance of erucic acid content was investigated in six backcross populations derived from three parental strains. The "zero erucic" strains were derived from two distinct sources and the "high erucic" strain was representative of Nugget. The erucic acid contents of the seed oils from both "zero erucic" strains were less than one per cent and the mean of the erucic acid content for the "high erucic" strain was 34.3% with a range from 31% to 38% (Figure 1, Table IV).

If inheritance is conditioned by an additive two gene system as reported by Harvey (8) then the backcross population from "zero erucic" from Liho by F_1 can be expected to fall into three classes with means of "zero", 8.6 and 17.2% erucic acid. (Assuming equal gene action, each gene would contribute one quarter of the value for the "high" parent (34.3%).) Three such classes were recovered (Fig. 1) with actual means "zero", 7.4 and 16.8% which are in good agreement with the expected values. The ranges of variation for the "high erucic" parent and for the 7.4 and 16.8% classes, 7, 7 and 8% respectively, are essentially equal.

If an additive two gene system is operating the indi-

Figure 1. Distribution of individual plants from parent and backcross populations based on erucic acid content of seed oil.

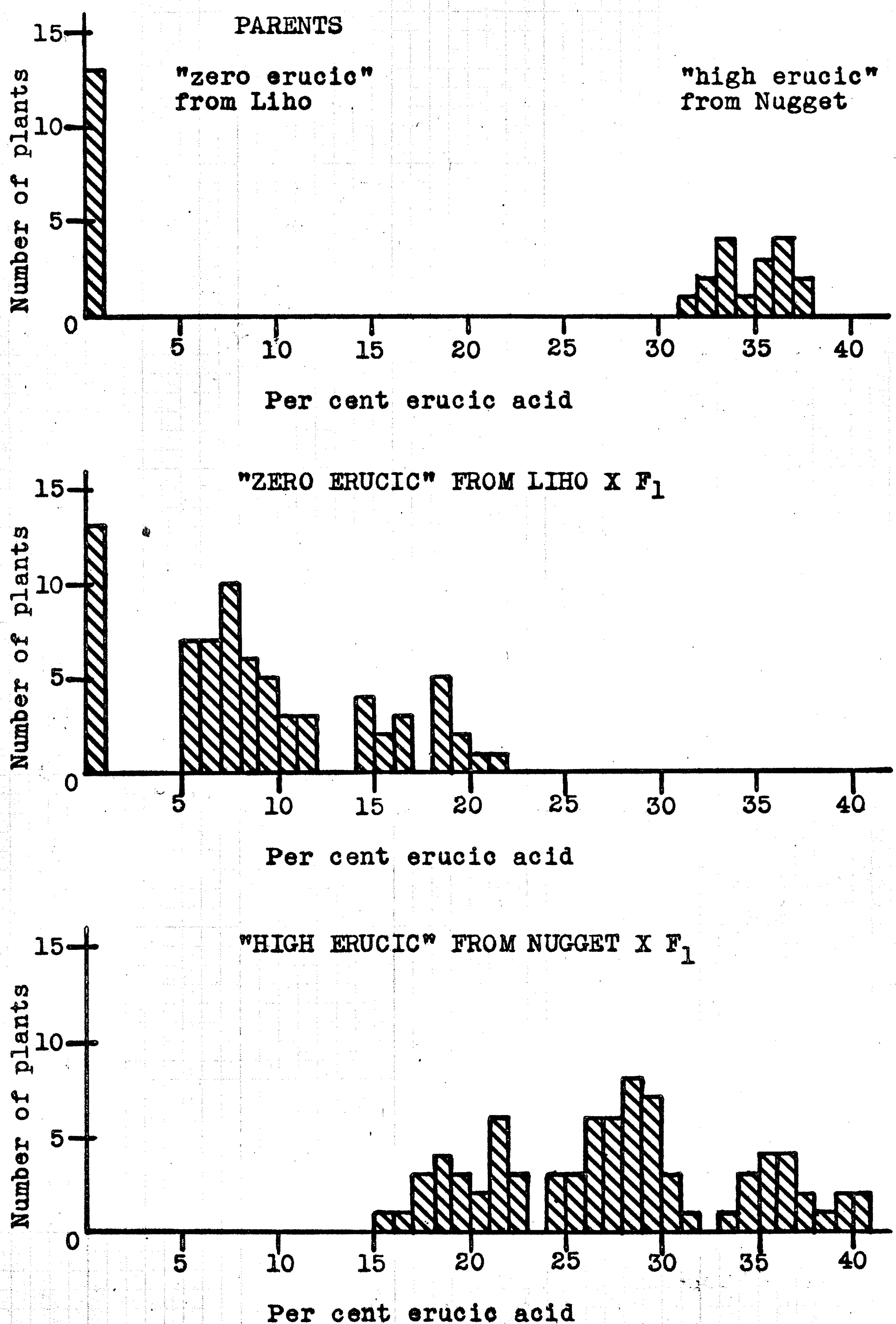


TABLE IV

POSTULATED GENOTYPES, ACTUAL AND EXPECTED MEANS, EXPECTED RATIOS AND RANGE OF ERUCIC ACID VALUES OF PARENT AND BACKCROSS POPULATIONS

			Per cent erucic acid					
			Liho x Nugget			Budapest x Nugget		
Parent or backcross population	Genotype	Expected ratio	Predicted mean	Mean	Range	Predicted mean	Mean	Range
"Zero erucic"	$e_1e_1e_2e_2$			0	0		0	0
"High erucic"	$E_1E_1E_2E_2$			34.3	31--37		36.5	33--39
"Zero erucic" x F_1	$e_1e_1e_2e_2$	1	0	0	0	0	0	0
	$E_1e_1e_2e_2$ $e_1e_1E_2e_2$	2	8.6	7.4	5--11	9.1	8.8	5--12
	$E_1e_1E_2e_2$	1	17.2	16.8	14--21	18.3	18.5	15--21
"High erucic" x F_1	$E_1e_1E_2e_2$	1	17.2	19.3	15--22	18.3	18.1	14--21
	$E_1E_1E_2e_2$ $E_1e_1E_2E_2$	2	25.7	27.4	24--31	27.4	27.3	22--32
	$E_1E_1E_2E_2$	1	34.3	36.3	33--40	36.5	35.6	33--40

vidual plants in the "zero", 7.4 and 17.2% classes should occur in a 1:2:1 ratio respectively. Testing to a 1:2:1 ratio gave a chi-square of 2.0833 with a probability value between 0.250 and 0.500, indicating agreement with a 1:2:1 ratio (Table V).

According to the two gene hypothesis the backcross population from "high erucic" from Nugget x F_1 should also fall into three classes with means of 17.2, 25.7 and 34.3% erucic acid content. Three distinct classes were recovered (Fig. 1) with means of 16.8, 27.4 and 36.3% which are in agreement with the predicted values. The range of erucic acid content for each of the three classes was eight per cent. These ranges are essentially equal to the range for the "high" parent, which was seven per cent. Individuals in each of these classes should occur in a 1:2:1 ratio respectively. The chi-square of 0.7215 with a probability value between 0.500 and 0.750 indicates good agreement with a 1:2:1 ratio. These results very distinctly support the hypothesis that erucic acid content of seed oil is controlled by an additive two gene system.

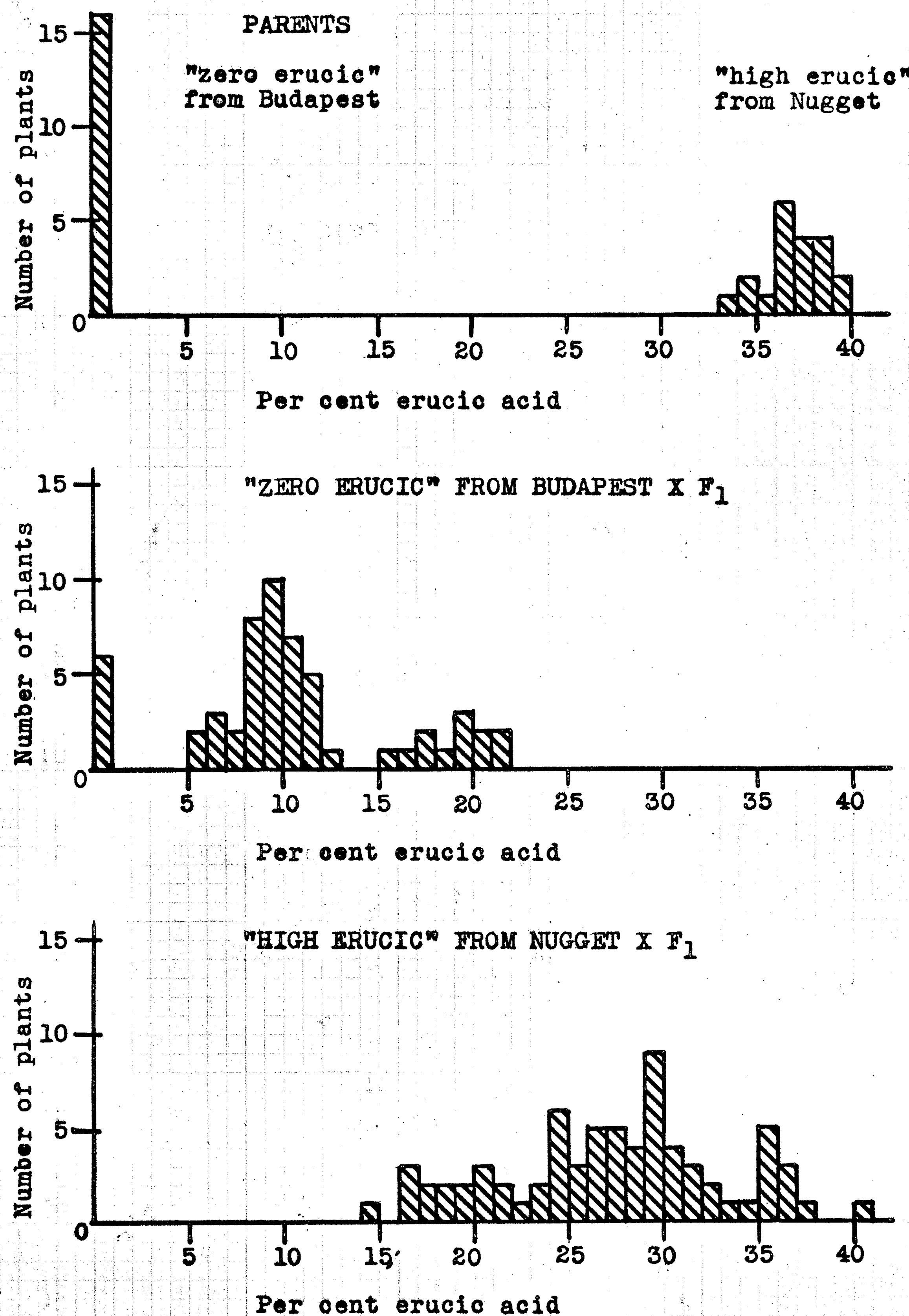
The backcross populations from the cross of "zero erucic" from Budapest by "high erucic" from Nugget produced similar results (Fig. 2) as those from Liho sources. The population from the backcross of "zero erucic" from Budapest was divided into three distinct classes with means of "zero",

TABLE V

SUMMARY OF CHI-SQUARE TESTS FOR INHERITANCE OF ERUCIC ACID

Backcross population	Population distribution	Assumption	X^2	P
"Zero erucic" from Liho x F_1 (n = 72)	13:41:18	1:2:1	2.0833	0.25--0.50
"High erucic" from Nugget x F_1 (n = 79)	23:37:19	1:2:1	0.725	0.50--0.75
"Zero erucic" from Budapest x F_1 (n = 57)	6:39:12	1:2:1	9.000	0.01--0.025
	6:51	1:3	5.3684	0.01--0.025
	45:12	3:1	0.4737	0.25--0.50
	6:39:12	1:6:1	3.8420	0.10--0.25
	6:51	1:7	0.2030	0.50--0.75
	45:12	7:1	3.8120	0.025--0.05
"High erucic" from Nugget x F_1 (n = 71)	15:44:12	1:2:1	4.1831	0.10--0.25
	15:56	1:3	0.5681	0.25--0.50
	59:12	3:1	2.4836	0.10--0.25
	15:44:12	1:6:1	6.9342	0.025--0.050
	15:56	1:7	4.8914	0.025--0.050
	59:12	7:1	1.2575	0.25--0.50

Figure 2. Distribution of individual plants from parent and backcross populations based on erucic acid content of seed oil.



8.8 and 18.5% which correspond to the predicted means of "zero", 9.1 and 18.3%. The ranges of the parental, 8.8 and 18.5% classes are 7, 8, and 7% respectively, and are essentially equal. If an additive two gene system is operating the individuals in the classes should occur in a 1:2:1 ratio. The chi-square value of 9.0000 with a probability value between 0.010 and 0.025 indicates poor agreement with a 1:2:1 ratio. Combining the "zero" and 8.8% classes and testing to a 3:1 ratio resulted in a chi-square of 0.4737 having a probability value between 0.250 and 0.500, which indicates agreement with a 3:1 ratio. Combining the 8.8 and 18.5% classes and testing to a 1:3 ratio resulted in a chi-square value of 5.3684 with a probability value between 0.010 and 0.025 which indicates poor agreement with a 1:3 ratio.

The preceeding results indicate a deficiency in the "zero" class. Difficulty in obtaining self-pollinated seed in some populations was previously mentioned. Self-pollinated seed was very difficult to produce in this population and it is possible that a degree of self-sterility was associated with the "zero erucic" class.

An additive three gene system would result in a 1:6:1 ratio, assuming that only the two extreme classes could be separated from the intermediate class. Testing to a 1:6:1 ratio resulted in a chi-square of 3.8420 with a probability value between 0.100 and 0.250 which indicates fair agreement with a 1:6:1 ratio. The agreement with a 1:6:1 ratio contradicts better agreement with a 3:1 ratio.

Combining the "zero" and the 8.8% classes and testing to a 7:1 ratio produced a chi-square of 3.8120 with a probability value between 0.025 and 0.050 which indicates poor agreement with a 7:1 ratio. Combining the 8.8 and 18.5% classes and testing to a 1:7 ratio resulted in a chi-square of 0.2030 with a probability value between 0.500 and 0.750, which is very good agreement with a 1:7 ratio. The agreement with a 1:7 ratio would be expected since there appears to be a deficiency in the "zero erucic" class. The deficiency in this class probably explains the conflicting evidence obtained from tests to ratios expected for a two or three gene system.

Some overlapping of classes was observed in the population from the backcross of "high erucic" from Nugget by F_1 (Fig. 2). The class with predicted mean of 18.3% was separated from the population on the basis of the range of the 18.5% class from the backcross of "zero erucic" from Budapest by F_1. The class with predicted mean of 36.5% was separated from the population on the basis of the range of the "high erucic" parent. The remaining individuals formed the intervening class with predicted mean of 27.4%. The actual means of the three classes were 18.1, 27.3 and 35.6% which correspond to the predicted means of 18.3, 27.4 and 36.5% respectively. If the additive two gene system hypothesis is correct, the individuals in the classes should occur in a 1:2:1 ratio. The chi-square of 4.1831 with a probability value between 0.10 and 0.25 indicates agreement with a 1:2:1 ratio. Misclassification of several individuals would not affect the probability range. Combining classes and testing to a 1:3 ratio gave a probability value between 0.250 and 0.50 and testing to a 3:1 ratio gave a probability value between 0.10 and 0.25. These results support the two gene hypothesis.

Testing to a 1:6:1 and 1:7 ratio gave a probability value between 0.025 and 0.050 which indicates poor agreement with these ratios. Testing to a 7:1 ratio gave a chi-square of 1.2575 with a probability value between 0.25 and 0.50, which indicates good agreement with a 7:1 ratio. These results support the two gene hypothesis rather than a three gene hypothesis.

The genetic interpretation involving postulated genotypes, expected ratios, actual and expected means and ranges of erucic acid values for the classes are summarized in Table IV (page 28). The chi-square tests and probabilities are summarized in Table V (page 30). The similarity of the means of the classes from the two different sources, the equality of class ranges, and particularly the agreement of the actual and predicted class means, supports the two gene hypothesis for inheritance of erucic acid in backcross populations derived from crosses involving the strain "zero erucic" from Budapest. While most of the evidence supports the hypothesis of an additive two gene system for the inheritance of erucic acid content of seed oil, the possibility of a three gene system in _B. napus_ from Budapest has not been entirely eliminated.

The erucic acid values for all cross-pollinated seed and all backcross populations (total of eighty-eight individual plants) from crosses between the "zero erucic" parents were "zero erucic". Lack of transgressive segregation in backcross populations derived from crosses between the two different sources of "zero erucic" (from Liho and from Budapest) and evidence for a two gene system in both sources suggests two common gene loci are involved in each of the two strains.

III. EICOSENOIC ACID

The inheritance of eicosenoic acid content of seed oil was investigated in the same populations which were used to study erucic acid. The mean of the eicosenoic acid content of the seed oils from twelve progeny of "low eicosenoic" from Liho was 0.8% with a range of 0.0--2.0% (Fig. 3, Table VI). The mean value of the sixteen progeny of "high

Figure 3. Distribution of individual plants from parent and backcross populations based on eicosenoic acid content of seed oil.

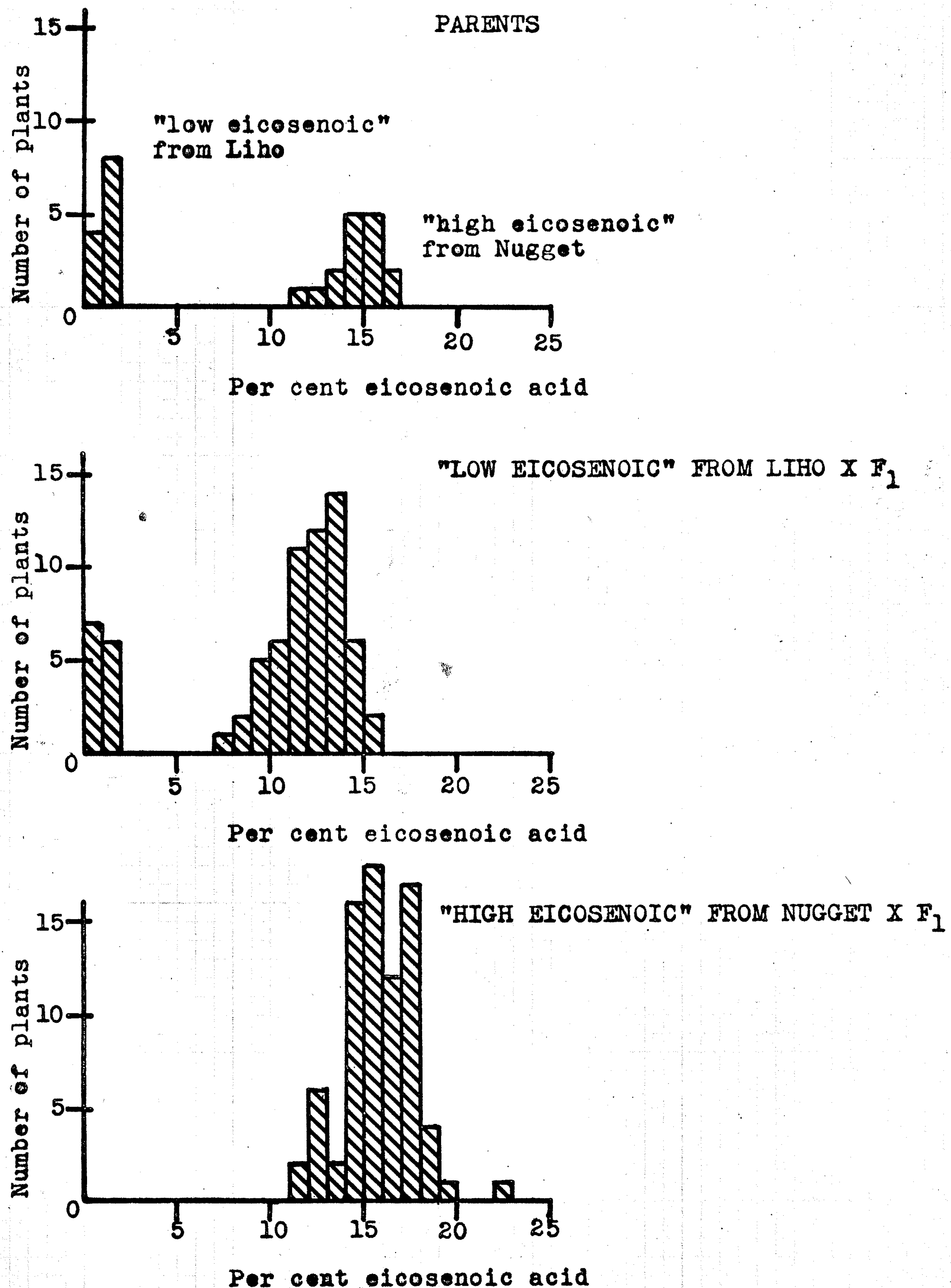


TABLE VI

POSTULATED GENOTYPES, EXPECTED RATIOS, MEANS AND RANGES OF EICOSENOIC ACID CONTENTS OF PARENT AND BACKCROSS POPULATIONS

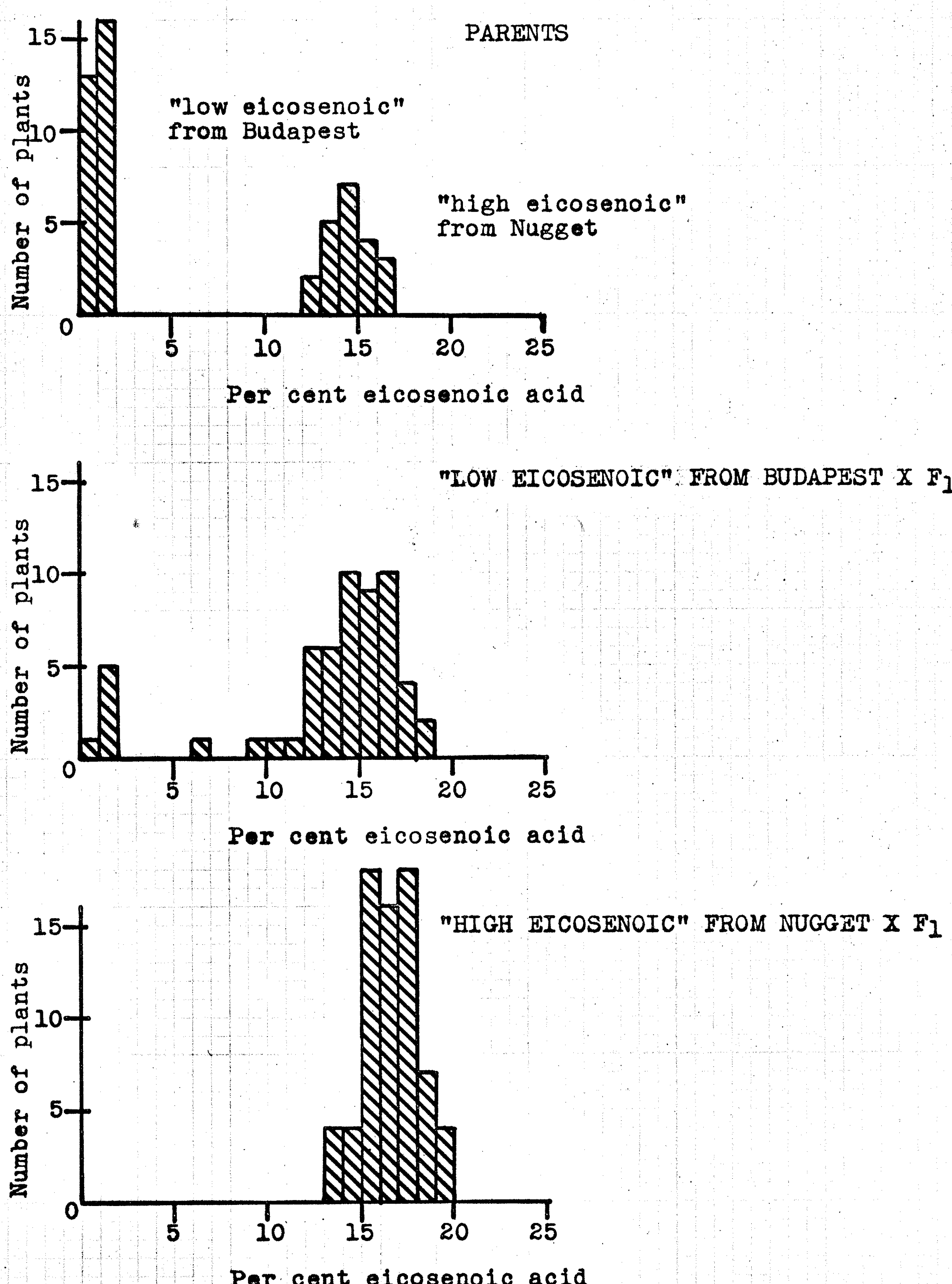
Parent or backcross population	Postulated genotypes	Expected ratios	Per cent eicosenoic acid			
			Liho x Nugget		Budapest x Nugget	
			Mean	Range	Mean	Range
"Low eicosenoic"	$e_1e_1e_2e_2$		0.8	0--2	0.6	0--3
"High eicosenoic"	$E_1E_1E_2E_2$		14.1	11--16	13.6	12--16
"Low eicosenoic" x F_1	$e_1e_1e_2e_2$	1	0.5	0--2	0.8	0--2
	$E_1e_1e_2e_2$ $e_1e_1E_2e_2$ $E_1e_1E_2e_2$	3	11.6	7--15	14.1	6--18
"High eicosenoic" x F_1	$E_1e_1E_2e_2$ $E_1E_1E_2e_2$ $E_1e_1E_2E_2$ $E_1E_1E_2E_2$		15.3	11--22	16.1	13--19

eicosenoic" from Nugget was 14.1% with a range of 11--16%. The population from the backcross of "low eicosenoic" from Liho by F_1 fell into two distinct classes with means of 0.5 and 11.6%. The number of plants in these classes, thirteen and fifty-nine respectively, was unequal. Evidence from cross-pollinated seed indicated dominant gene action for the gene or genes conditioning eicosenoic acid content. The individuals in the 0.5 and 11.6% classes were tested to a 1:3 ratio giving a chi-square of 1.8519 with a probability value between 0.100 and 0.250 which indicates agreement with a 1:3 ratio. The backcross population of "high eicosenoic" from Nugget by F_1 produced only one class which had a mean of 15.3%, further indicating dominant gene action. A two gene system must be involved in the genetic control of eicosenoic acid content to produce a 1:3 ratio where the complete recessive produces zero to three per cent eicosenoic acid and one or more dominant genes in the genotype produces at least seven per cent eicosenoic acid. The 11.6 and 15.3% high eicosenoic classes from the two different backcross populations with ranges of eight and eleven per cent respectively differ from each other and from the "high eicosenoic" parent which had a mean of 14.1% and range of only four per cent. This evidence of transgressive segregation suggests modifying factors.

The backcross populations from the cross of "low eicosenoic" from Budapest by "high eicosenoic" from Nugget produced similar results (Fig. 4 and Table VI, page 37). Two distinct classes with means of 0.8 and 14.1% occurred in the backcross population of "low eicosenoic" from Budapest by F_1. Testing the individuals in the classes to a 1:3 ratio resulted in a chi-square of 5.2684, having a probability value between 0.010 and 0.025, which indicates poor agreement with a 1:3 ratio. When tested to a 1:7 ratio as would be expected from a three gene system, a chi-square of 0.2030 with a probability value between 0.500 and 0.750 resulted, indicating agreement with a 1:7 ratio. These results are expected since the "low eicosenoic" and "zero erucic" values always occurred together; a low value of one never occurred when the value of the other was high. Since the "low eicosenoic" and "zero erucic" types represent the same plants, the deficiency of "zero erucic" plants noted in the erucic acid study of this population also is a deficiency of "low eicosenoic" plants. Since most of the evidence supports a two gene hypothesis for the inheritance of erucic acid content, a two gene system probably also applies to the inheritance of eicosenoic acid control.

The mean of the progeny from the "high eicosenoic" parent was 13.6% compared with 14.1 and 16.1% for the "high eicosenoic" classes from the two backcross populations. The range of the "high eicosenoic" parent was four per cent

Figure 4. Distribution of individual plants from parent and backcross populations based on eicosenoic acid content of seed oil.



compared with twelve and six per cent for the "high eicosenoic" classes. The differences in means and ranges indicate transgressive segregation and suggests modifying factors.

The zero to three per cent eicosenoic acid contents are associated exclusively with "zero erucic" content and the six to twenty-two per cent eicosenoic acid contents are exclusively associated with five to forty per cent erucic acid values. This association coupled with evidence that two major genes control erucic and eicosenoic acid content indicates that the same genes that control erucic acid content also control eicosenoic acid content. The evidence shows that the same gene system acts in a dominant manner with respect to eicosenoic acid but in an additive manner for erucic acid. The genetic interpretation involving postulated genotypes, expected ratios and actual means and ranges are summarized in Table VI (page 37). A summary of chi-square tests and probabilities are presented in Table VII.

IV. OLEIC ACID

The inheritance of oleic acid content of seed oil was investigated in the same populations which were used to study erucic acid. The progeny of each parental strain have a very wide range of oleic acid values (Table VIII). Three classes are expected in each backcross population

TABLE VII

SUMMARY OF CHI-SQUARE TESTS FOR INHERITANCE

OF EICOSENOIC ACID

Backcross population	Population distribution	Assumption	X^2	P
"Low eicosenoic" from Liho x F_1 (n = 72)	13:59	1:3	1.8519	0.10-0.25
"High eicosenoic" from Nugget x F_1 (n = 79)		1	0	1.0
"Low eicosenoic" from Budapest x F_1 (n = 57)	6:51	1:3	5.3684	0.01-0.025
	6:51	1:7	0.2030	0.50-0.75
"High eicosenoic" from Budapest x F_1 (n = 71)		1	0	1.0

TABLE VIII

DISTRIBUTION OF INDIVIDUAL PLANTS FROM PARENT AND BACKCROSS POPULATIONS BASED ON THE OLEIC ACID CONTENT OF THE SEED OIL

Parent or backcross population	Per cent oleic acid																					
	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	Mean
Liho											1	2	4	3	1	1	1						54.0
Nugget		1	7	6	2																		23.4
Liho x F_1					2	5	4	5	8	13	11	9	2	2	1	3	6	1					45.3
Nugget x F_1	2	6	14	18	12	12	10	3	3														27.0
Budapest																	1		3	8	15	2	74.4
Nugget		9	10	2																			20.7
Budapest x F_1							3	1	8	2	8	13	11	2	2	1	1		1	5			50.1
Nugget x F_1		2	7	19	20	6	9	6	1		1												28.2
Liho												1	7	9	7								54.6
Budapest																1	3	5	2	1			66.8
Liho x F_1													1	5	15	16	14	6					58.8
Budapest x F_1															1	9	9	5	5	3			65.4

derived from crosses involving "high erucic" from Nugget, due to the correlation of oleic and erucic acid (Craig (3)). The backcross populations did not separate into distinct classes except in the backcross population resulting from "zero erucic" from Budapest by F_1 (Budapest by "high erucic" from Nugget) where well defined "high oleic" class occurred. The "high oleic" plants were the "zero erucic" plants of this population which was expected.

In each backcross population derived from crosses involving "high erucic" from Nugget there is an indication of three classes. The oleic acid classes are less distinct than those obtained for erucic because of several possible factors. The interrelationship of oleic and linoleic acid and of oleic, eicosenoic and erucic acid probably added variation to the oleic acid contents. The degree of interaction between the genotype of the maternal sporophyte and the genotype of the developing seed is an unknown factor. The wide range of oleic acid values of progeny from parental strains itself indicates the difficulty of classifying genotypes by the oleic acid content of the seed oil.

V. LINOLEIC ACID

The inheritance of linoleic acid content of seed oil was studied in the same populations which were used to study erucic acid. The results of the linoleic acid study could not be readily analyzed because no indication of classes was present in the backcross populations (Table IX). The lack of classes suggests quantitative inheritance, but was most probably due to the wide ranges of linoleic acid values within parental strains relative to the differences between parental strains. The wide ranges of linoleic acid values were probably due to the same factors that affect oleic acid content of seed oil.

TABLE IX

DISTRIBUTION OF INDIVIDUAL PLANTS FROM PARENT AND BACKCROSS POPULATIONS BASED ON THE LINOLEIC ACID CONTENT OF THE SEED OIL

Parent or backcross population	Per cent linoleic acid																		
	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	Mean
Liho								1	1	2	4	5	1						28.6
Nugget			1	11	3	1													14.9
Liho x F_1				1	3	11	25	23	7	2									21.0
Nugget x F_1			2	23	41	10	3												16.1
Budapest	4	18	8	1															10.8
Nugget			1	10	10														15.5
Budapest x F_1	2	18	21	11	5														12.5
Nugget x F_1		1	13	27	21	8	1												15.1
Liho									1	9	11	3	1						28.1
Budapest		1	1	2	1	5	1												16.6
Liho x F_1						5	13	10	11	14	3	1							23.4
Budapest x F_1		2	1	3	6	5	11	3											18.0

SUMMARY

The effect of pollen source and maternal parent on composition of seed oil was studied in diallel crosses involving four parental strains; "zero erucic" from Liho, "zero erucic" from Budapest, "zero erucic, high linoleic" from Liho and "high erucic" from Nugget. All strains were of the species B. napus.

The fatty acid composition of oil from cross- and self-pollinated seed indicated that erucic acid content is determined by the genotype of the developing seed and that the erucic acid value of cross-pollinated seed is intermediate to the two parental values.

Eicosenoic acid content of seed oil is also determined by the genotype of the developing seed but the maternal sporophyte may exert some effect on eicosenoic acid. The genes for eicosenoic acid content act in a dominant manner.

The oleic acid content of seed oil is influenced by the maternal parent except in crosses involving differences in erucic acid content where the maternal effect is not evident. The maternal sporophyte definitely influences the linoleic acid content and appears to influence linolenic acid content.

The inheritance of erucic, eicosenoic, oleic and linoleic acids was investigated in six backcross populations

derived from crosses involving three parental strains; "zero erucic" from Liho, "zero erucic" from Budapest and "high erucic" from Nugget. Conclusive evidence was obtained that in both "zero erucic" strains, erucic acid content of seed oil is determined by a two gene system acting in an additive manner. Eicosenoic acid content is controlled by a two gene system acting in a dominant manner. The regular association of eicosenoic and erucic acid values indicates that the same genes control the content of both fatty acids. The same gene system which acts in an additive manner with respect to erucic acid, acts in a dominant manner for eicosenoic acid.

Inheritance of oleic and linoleic acid is complex due to the interrelationship of oleic and linoleic, and of oleic, eicosenoic and erucic acids. The inheritance of these fatty acids may be further complicated by interaction between the genotype of the maternal sporophyte and the genotype of the developing seed.

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