INHERITANCE OF OLEIC TO LINOLEIC FATTY ACID RATIO IN PEANUTS, *ARACHIS HYPOGAEA*, L.

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CHAPTER I

INTRODUCTION

The cultivated peanut, Arachis hypogaea L., Leguminosae family is grown on approximately forty-five million acres in the world. Since the peanut cotyledons are rich in protein, oil and vitamins, peanuts are a favorite food for man as well as feed for poultry and livestock in many areas of the world.

Peanut seeds are composed of approximately equal weight of fatty and nonfatty constituents, the relative amounts of each depending upon variety and quality of the peanuts. Peanut oil is made up of at least eight major fatty acids plus many minor ones. A great part of the peanut oil is unsaturated fatty acids, of which 40 to 45 percent is oleic acid and 30 to 35 percent is linoleic acid. Commercially, Spanish peanut seed contain a relatively higher percentage of linoleic acid and a lower percentage of oleic acid than the Virginia type. Pickett and Holley (47) and Holley and Hammons (27) reported that oleic and linoleic glyceride values showed very little variation due to environmental influence. However, knowledge of the inheritance of peanut fatty acid composition is lacking.

The stability of peanut oil could possibly be improved considerably if the fatty acid pattern in this crop could be changed through plant breeding. Since linoleic acid is one of the factors primarily responsible for "off-flavors" or "rancidity" in peanut oil and other peanut

products, diminishing the content of this acid could improve the shelf-life of peanut products considerably.

Peanut cultivars with a range of "high" to "low" ratios of oleic acid to linoleic acid in the peanut seed oil have been collected. Crosses with some of them have been made and F_2 and F_3 segregating populations were available from the greenhouse and the field for analyses.

The objectives of this research were to study: (1) the environmental influence on the O/L ratio of peanut seed oils; (2) the maternal effects on the O/L ratio; (3) the mode of inheritance of the ratio of oleic acid to linoelic acid in peanuts; and (4) the association between O/L ratio and peanut butter shelf-life for ten selected peanut cultivars.

CHAPTER II

LITERATURE REVIEW

I. Fatty Acid Composition of Peanut Seed Oil

Iverson, et al. (30) examined the fatty acid composition of oil extracted from roasted Virginia peanut samples. They found that the mean composition of long-chain fatty acids was as follows: 1.23, 1.16, 3.12, and 1.70 percent, respectively, for arachidic, eicosenoic, behenic, and lignoceric. The average composition for the major fatty acids was 11.2, 2.85, 49.40, and 29.20 percent, respectively, for palmitic, stearic, oleic, and linoleic. Approximately 0.4 percent hexacosanic (26:0) was present in the oils as well as trace amounts of odd chain-length saturated fatty acids (13-27 carbon atoms). They also detected a small amount of capric (10:0) and confirmed the presence of caprylic (8:0), lauric (12:0) and palmitoleic (16:1) acids, but no linolenic acid was detected. Worthington and Holley (58) later found that linolenic acid was present in the oil of all varieties they used but at a level not exceeding approximately 0.04 percent of the total fatty acids. However, the oil from immature peanuts showed a somewhat higher content of linolenic acid, particularly in tissue other than the cotyledon.

Iverson, et al. (30) found that roasting the peanut did not change the fatty acid composition of the extracted oil and the fatty acid composition of commercial peanut oils is similar to the composition of

oils from roasted peanuts.

Jamieson, et al. (31) found glycerides of oleic acid lower and linoleic higher in oil from Spanish peanuts than in oil from Virginia peanuts. They also found glycerides of the saturated fatty acids higher in the Spanish oil than in that from the Virginia.

Higgins, et al. (23) analyzed 24 hybrid selections, with Spanish and Southeastern Runner as checks. Oils from the hybrid selections were variable in the percentages of oleic and linoleic fatty acids, while the proportions of oleic and linoleic glycerides in oil from the Spanish and Southeastern Runner checks were similar to those reported by Jamieson, et al. (31) for Spanish and Virginia peanuts. Using gas chromatography, Fore, et al. (18) showed that (a) peanuts contain from 45 to 49 percent oil, made up of at least eight nutritionally essential fatty acids; (b) peanut oil contains 76 to 86 percent unsaturated fatty acids, of which 40 to 45 percent is unsaturated oleic acid, and 30 to 35 percent is polyunsaturated linoleic acid; (c) Spanish type peanuts contain a high percent of saturated fatty acids, giving a wide variation in kind of fatty acids; (d) Runner and Virginia types of peanuts are higher in mono-unsaturated fatty acids, chiefly oleic; and (e) there is no apparent relation between polyunsaturated fatty acids and methods of harvesting and curing, or types of damage.

Holley and Hammons (27) studied 67 strains of peanuts and found that the correlation coefficient of oleic to linoleic acid was -0.988. Waller, et al. (55) also reported a negative correlation of -0.9645 between these two acids based upon the half-seed analysis.

II. Distribution of Fatty Acids in Seed

Bauman, et al. (2,3) suggested that improvement in quality of grain crops by screening of genotypes on a single seed basis may be more efficient than other methods using one or more plants as units of selection. As Singh, et al. (51) pointed out, however, the success of the single seed approach depends upon the development of techniques for measuring the desired attribute while retaining viability of the seed. Whether a sample of a seed fat does represent the genotype or not is an important question.

Kartha (35) showed that the variations in oil content and iodine value occur in systematic patterns with reference to the center of the seed or in some cases the center of each cotyledon. The patterns differ with different varieties of seed, on some from the center to the periphery, in others, along the vertical. The oil content decreases, while the iodine value increases, from the center to the periphery in peanuts. He found that oil content variations, downward along the vertical axis in peanut seeds, were 50.4, 56.3, and 53.7 percent for top, middle, and bottom, respectively. Iodine value variations were 92.2, 85.5, and 90.8 for top, middle and bottom, respectively.

In the soybean, Singh, et al. (51) found that protein content of the seed coat, root-shoot axis and cotyledon differed markedly. The order of decreasing protein for these seed parts was as follows: cotyledon $\geq$ (greater than or equal to) root = shoot axis $\geq$ seed coat. They also observed that protein content showed a slight gradient across the cotyledon, with the highest percentage occurring in the region adjacent to the root-shoot axis and a decrease in protein content from the adjacent region to the extreme opposite region. They indicated

that this technique, however, will be useful to the soybean breeder only if heritability for differences in protein content among seeds from a heterozygous plant is relatively high. If the protein content of a seed is determined by the genotype of the sporophyte bearing the seed, no advantage exists in single seed analysis.

Fedeli, et al. (16) extracted lipids from three different parts of peanut seeds (cotyledons, germs, and hulls) and examined their fatty acid and unsaponifiable composition. They found that germs are richer in palmitic and linoleic acids, while cotyledons and hulls have more oleic acid than germs. Germs have higher values for the more unsaturated glycerides than either hulls or cotyledons.

III. Environmental Influence on the Fatty Acids

Hilditch (24) reported that in seeds of the same species, the relative proportions of oleic and linoleic acids may vary considerably, and such variation is conditioned mainly by the temperature of the locality where the seed ripens. Low temperature (and rate of development of the seed) favor the production of more unsaturated mixture of acids, and conversely.

Pickett and Holley (48) found that several strains of Spanish peanuts in four or more crop years at two locations showed very little variation in calculated oleic and linoleic glyceride values. Holley and Hammons (27) also confirmed this characteristic. They concluded that genetic characters appear to be fixed in 26 peanut cultivars. However, there are exceptions, such as Ga. 207-3-4, which still exhibit instability as reported by Pickett and Holley years ago (47). Stansbury, et al. (53) reported that environment affected composition

after observing higher oil and protein content of Spanish seed from Texas compared to those from Alabama and Georgia. Hilditch and Williams (25) pointed out that, although peanut oils from the somewhat cooler climates of China and Argentina also belong to the group richest in linoleic acid, the high linoleic acid figures for peanut oils from Tanganyika suggest that, in this instance, cooler climates with slower development of seeds are not the sole causes of the production of the more unsaturated peanut oils. They concluded that, besides varietal differences and the influence of temperature during ripening of the nuts, other factors contribute materially to the observed differences in composition of peanut oils.

Howell and Collins (29) found that the linoleic and linolenic acid percentages of soybean oil were significant, and were inversely correlated with daily maximum temperatures during seed development. They indicated that the correlation of linolenic acid and temperature was closer than that of linoleic acid and temperature. Changes in environmental factors, such as photoperiod, light intensity and quality, nitrogen, phosphorus, potassium and sulphur nutrition, or the addition of manure or plant residues, had little or no effect on the levels of these fatty acids.

Collins and Sedgwick (8) studied the environmental effects on soybean oil composition in the United States and found that the oil produced in northern areas was high in linolenic acid and low in linoleic acid when compared to southern grown material.

Ladd and Knowles (39) found that growing temperature had a profound effect on the seed oils of safflower. Cooler growing conditions lowered the relative amounts of oleic and stearic acid and increased

linoleic acid while no significant effects on palmitic acid were observed.

In flax seeds, Dillman and Hopper (13) reported that the correlations between the percentages of the different fatty acids in the oils and July temperature showed that temperatures during the oil formation period are negatively correlated with linolenic acid and positively correlated with the saturated and oleic acids. They pointed out that the coefficients were small, but did show the influence of temperature.

Painter and Nesbitt (46) found that flax seeds showed not only a wide range in the amount of oleic, linoleic, and linolenic acids in oils from each variety grown at different locations, but also in oils of a single variety grown at the same location in different crop years. McGregor and Carson (43) found that differences in iodine number and linolenic acid due to environment were greater than differences between varieties grown in the same location. They also found that linseed flax grown in northern areas had a higher oil and linolenic acid content than flax grown farther south in Canada. Yermanos and Goodin (60) applied differential temperatures (constant 50, 60, 70, and 80^{o} F) before and after initiation of floral primordia in two varieties of seed flax and found that preflower treatments caused significant differences in vegetative development but did not affect fatty acid composition of the seed oil of either variety. Post-flower-temperature, however, affected fatty acid composition of the oil drastically. Linolenic acid decreased and oleic acid increased as post-flowering temperatures increased from 50 to 80^{o} F, but linoleic acid was not affected significantly. Yermanos, et al. (61) found that seed oil content and iodine

value of oil from seed flax and safflower were not affected by iron chelate except in one case when an increase in iodine value was associated with a decrease in seed yield. They could not show a possible influence of iron chelates on fatty acid composition of vegetable oils. They also found that nitrogen fertilization depressed the iodine value of oil and seed oil content but increased seed yield.

Craig and Wetter (11) found that rape seed fatty acid composition lacked significant variation between stations and suggested that varietal effects were greater than environmental. Craig (10) later, however, found significant differences between stations for all fatty acids and between varieties for palmitic, stearic, oleic, linoleic, eicosenoic, and erucic acids.

In their study on factors affecting oil content and oil composition of corn grain, Jellum and Marion (33) found that oil content and quality were not greatly affected by different dates of planting. Ear position had no affect on palmitic, stearic, and linolenic acids. Although not always significant, the oil from the first ear was always higher in oleic acid and lower in linolenic acid than that from the second ear. Year, location, and hybrid effects were highly significant for all characters. First and second order interactions were generally significant. Hybrids had a greater influence on the characters studied than any of the environmental factors.

IV. Maternal Influence on the Fatty Acids

Techniques for rapid analysis of fatty acid composition and oil content of peanut seeds are now available (63). Furthermore, these techniques do not destroy the seeds. With these techniques, genetic

and selection studies on oil quality are possible on single seeds. Before these techniques can be used effectively, one must determine if fatty acid composition is controlled by the genotype of the seed or by the genotype of the maternal sporophyte.

Yermanos and Knowles (62) found that the fatty acid composition of flax seed oil is controlled largely by the genotype of the seed itself, and only to a limited extent by the genotype of the maternal sporophyte.

Knowles and Hill (36) reported that the oil quality of a safflower seed is determined by its genotype, not by the genotype of the maternal plant. Ladd and Knowles (39) confirmed this conclusion. However, Knowles and Mutwakil (37) and Knowles and Hill (36) showed that the control of the oil content of the safflower seed was determined by the genotype of the maternal parent.

Jellum (32) used gas-liquid chromatography to determine the fatty acid composition of maize oil for certain inbred lines and reciprocal crosses and found that the maternal parent significantly influenced the fatty acid composition of hybrid corn embryos in certain reciprocal crosses, but not in others. Plant-to-plant reciprocal crosses were made by Garwood, et al. (19) between the maize strain Illinois High Oil and each of seven maize inbreds plus the strain Illinois Low Oil. Oil analyses showed that the direction of the cross caused the oil content of F_1 kernels to vary an average of 3 percent. Paternal effects were of similar magnitude. Because the largest cytoplasmic effect on oil was 0.14 percent, they concluded that the physiological influence of the female parent was responsible for the 3.00 percent maternal effect.

Reciprocal crosses between rape plants containing no erucic acid and normal high erucic acid indicated that the fatty acid composition was controlled by the genetic constitution of the developing embryo, rather than the maternal parent (15).

Recent studies (5,6,50) have shown that oil synthesis in soybean seed is determined largely by the genotype of the plant producing the seed rather than by the genotype of the seed even though oil synthesis takes place in the seed.

V. Inheritance of Fatty Acids

A. Soybean

Oil content of soybeans is controlled primarily by additive genetic effects (4,50), although partial dominance (50) and epistatic effects (20,21) have been shown. Heritabilities for oil content in F_2 generations of 0.34 to 0.59 have been reported (21) and in subsequent generations of 0.61 to 0.78 (17). A negative correlation between percent oil and percent protein has been observed by several investigators (17,34).

White, et al. (56) reported that crosses of soybean varieties and introductions "high" and "low" in linolenic acid indicated that inheritance of the fatty acid was quantitative rather than qualitative. Transgressive segregation, particularly to low values, was observed occasionally in the crosses.

B. Flax

Yermanos and Knowles (62) found that selfed and crossed seeds developing on the same flax plant did not have the same fatty acids

when the parents differed in the fatty acid composition of their seed oil. The relative proportions of the fatty acids in the oil of the crossed seed tended to be intermediate but closer to those of the female parent. They concluded that the fatty acid composition of seed oil in flax is determined largely by the genotype of the seed itself. They also suggested that the inheritance of fatty acids in flax is not conditioned by strong dominance and epistatic pressure.

Yermanos, et al. (59) found that polyploidy *per se* did not cause major changes in flax fatty acid composition but it tended to depress linoleic and increase oleic acid.

C. Rape

Downey and Harvey (15) found, in reciprocal crosses between plants of *Brassica napus* with seed oil containing 40 and 0 percent erucic acid, an erucic acid content of 22 to 24 percent in the crossed seeds. They suggested that dominant gene action was absent and that fatty acid composition of the oil was controlled by the developing embryo. Harvey and Downey (22) confirmed their earlier conclusion that erucic acid content in seed oil of plants of *Brassica napus* is governed by two alleles displaying no dominance and acting in an additive manner.

Dorrell and Downey (14) found that the erucic acid content in the seed oil from F_1 embryos of *Brassica campestris* was intermediate between the two parents. This also confirmed the hypothesis that erucic acid synthesis is controlled by a single non-dominant gene.

Olsson and Anderson (44) estimated the degree of heritability of the oil content in winter rape by determining the coefficient of correlation between mother plant and progeny and found all correlations

highly significant. Therefore, the variation in regard to oil content depends to a considerable extent upon genetic differences.

D. Safflower

Safflower oil characteristics are determined in large part by one gene, $\underline{ol}^1$, which is allelic to both the gene ol for high oleic acid low linoleic acid in the oil of India 57-147, and the gene Ol for low oleic acid and high linoleic acid in the oil of commercial varieties (26,36).

Ladd and Knowles (39) reported that differences in percentages of stearic acid in the seed oil of the safflower introductions Israel 55-46 (high), Russia 60-110 (high) and the cultivar 'US-10' (low), were determined by alleles at a single locus. Seeds of genotypes St St, St st, and st st have oils with respective stearic acid contents of 1.0 to 2.5, 2.5 to 5.0, and 5.0 to 12.0 percent. Increases in the percentage of stearic acid were accompanied by decreases in the relative amounts of linoleic acid, oleic acid or both linoleic and oleic acids. Palmitic acid was usually reduced slightly as stearic acid increased.

Knowles and Hill (36) suggested that if oleic acid is the precursor of linoleic acid, then the ol alleles control, in some way, the synthesis of linoleic acid from oleic acid.

E. Corn

Bauman, Conway, and Watson (2,3) reported that, based on nuclear magnetic resonance (NMR) measurement, the correlation between oil content of single kernels and their F_2 progeny in corn was highly significant ($r = +0.75$). In 6 F_3 families, the correlations of oil

content of kernels versus their progeny were all highly significant and varied from r = 0.54 to r = 0.84 (3). They concluded that the large differences in oil content among kernels from the same ear were heritable in the F_2 and also in the F_3 generation, when heterozygosity was considerably reduced.

Jellum (32) found that oleic and linoleic acids showed partial dominance, or heterosis, in the F_1 kernels when compared with the parental inbred lines. Poneleit and Alexander (49), based on the results obtained from backcross and F_2 populations, suggested monohybrid inheritance for oleic and linoleic acid content. Their results support the hypothesis that the low linoleic acid content is dominant to high and the low oleic acid is recessive to high. They also suggested that desaturation at the 12-13 position in oleic acids is under simple Mendelian control.

F. Peanuts

Mason and Matlock (42), based on data available from crosses between Krinkle (P-151) and other type peanuts, concluded that the ratio of oleic and linoleic acids was quantitatively inherited and controlled by multiple factors.

Jamieson, et al. (31) reported that oil in Spanish peanuts contained slightly less oleic acid and more linoleic acid than that from Virginia peanuts. Higgins, Holley, Pickett, and Wheeler (23) also found a wide variation in the linoleic and oleic acid contents of some selected strains of Spanish and Runner peanuts. They confirmed the conclusion made by Jamieson, et al. (31) and indicated that the difference also showed in selected hybrid strains. Holley and Hammons

(27) found that the correlation coefficient between oleic and linoleic acids was -0.988. Waller, et al. (55) reported a similar negative correlation of -0.9645 for oleic and linoleic acids when using the half-seed technique on individual kernels of different cultivars.

According to Holley and Hammons (27), the Spanish characteristics will be especially difficult to introduce into any cross designed to yield a low linoleic acid oil, since linoleic acid oils so far reported are from large seeded types which have other characteristics quite different from the Spanish.

VI. Peanut Oil Quality and Keeping-time

When pure fat and fatty materials are stored, their quality will decrease and the rate depends both upon their chemical composition and to what extent they are exposed to air, light and heat (28,41,47). When the unsaturated fatty acids are attacked by oxygen they undergo a series of reactions at their double bonds and bad tasting split products will accumulate. There are larger differences in the rate of oxidation of mono- to polyunsaturated fatty acid than those due only to their number of double bonds. The rate of oxidation of oleic acid: linoleic acid: linolenic acid is reported to approximate 1 : 30 : 80 at 37° C, that of methyl stearic to methyl oleate is reported to be 1/11 to 1 at 100° C (1). But while linoleic acid is a necessary component in vegetable fats for nutritional reasons (1,45), the absence of linolenic acid from edible oils is a very reasonable demand from the viewpoint of food technology.

Peanut oil is fairly stable in that the iodine number, saponification number, acetyl number and free-fatty acids do not change during

the treatment involved in the manufacture of peanut butter or salted peanuts (12). Oil from peanut cotyledons is more stable and has a better flavor than that from the hearts (12).

Pickett and Holley (47) reported that oil from Spanish peanuts is more susceptible to peroxide rancidity than that from either the Runner or Virginia types. They found that not only did the Spanish appear to develop rancidity by the taste test earlier than either the Runner or Virginia but also their peroxide values were higher. Fore, et al. (18) studied the factors affecting the stability of crude oils of 16 varieties of peanuts and found that the relative linoleic acid content of the oils is one of the major factors affecting the stability of the oils tested. They suggested that products made from peanuts containing oils of relatively low linoleic acid content and of high stability could have an increased shelf-life. They also found that, with the exception of the oils from Runner type peanuts, the tocopherol compositions of the oils did not vary significantly, either in the nature and distribution of individual tocopherols, or in total tocopherol contents. The enhanced stability of the oils from the Runner peanuts may be due in part to the higher tocopherol content of these oils.

Holley and Hammons (27) confirmed the conclusion reported by Fore, et al. (18) and by Pickett and Holley (47) that linoleic acid is the predominant factor in peanut oil keeping-time. They also found that the high protein years produced the poorest oil from the standpoint of oil keeping-time. The protein showed a highly significant negative correlation of -0.527 with oil keeping-time.

Holley and Hammons (27) established a formula to predict the shelf-life of the oil by measuring the linoleic acid, oleic acid and protein

content of peanuts. According to their formula, linoleic acid accounts for 85 percent of the variation associated with oil stability.

Mason and Matlock (42) found that two varieties, Early Runner (P-215) and Fla. 393 (P-960), had wide oleic-linoleic acid ratios, but showed lower CLER scores for roasted peanuts. In a shelf-life test of peanut butter, they found Ga. 186-28 (P-972) had a narrow oleic-linoleic acid ratio and the preference rank for the peanut butter dropped slightly between 0 and 30 days, then remained constant during the rest of the experiment for both shelf and freezer stored samples. The other varieties with narrow oleic-linoleic acid ratios, such as F-416-2 (P-938) and Argentine (P-2), showed a less desirable preference rank for the samples stored on the shelf compared to those stored in the freezer. Also, there was a remarkable change to a less desirable preference rank between 90 and 182 days of shelf storage. Both Early Runner and Fla. 393 had good preference rank scores for the peanut butter stored 182 days regardless of the storage, either on the shelf or in the freezer.

Worthington and Holley (58) showed that the linolenic acid content of peanut oil obtained from mature kernels is uniformly low and suggested that the tendency of oil of some peanut varieties to develop oxidative rancidity is not correlated with linolenic acid content.

Woodroof, et al. (57) used several means to prevent or retard the development of rancidity in peanut oil. They found refrigeration was most effective. No critical temperature was found, but the lower the temperature, the longer the shelf-life of the peanut oil. They also found that daylight would bleach peanut oil and accelerate rancidity slightly.

CHAPTER III

GENOTYPE X ENVIRONMENT INTERACTION IN O/L RATIOS OF PEANUT SEEDS

Phenotype reflects nongenetic as well as genetic influence on development. Furthermore, the effects of genotype and environment are not independent. The phenotypic response to a change in environment is not the same for all genotypes; the consequences of variation in genotype depend on the environment. Comstock and Moll (9) called this inter-play of the genetic and nongenetic effect on development a genotype-environment (GE) interaction.

It has been reported that the fatty acid composition of peanut seed oils are greatly influenced by the environment (25,53,63). Information is needed as to whether peanut varieties respond differently in their O/L ratios when grown under different environmental conditions, and if so, how important such cultivar x environment interaction may be in an inheritance study and breeding program on the O/L ratios. The present study is designed to obtain estimates of the magnitudes of the cultivar x location, cultivar x year, and cultivar x year x location interactions in certain cultivars in 1969 and 1970 near Stillwater and Perkins, Oklahoma.

I. Materials and Methods

The materials selected for the present study are listed in Table I.

TABLE I

TEN PEANUT CULTIVARS CHOSEN FOR THE GENETIC STUDIES ON THE O/L RATIOS

P-No.	Cultivar	Market Type	Reported O/L	Stillwater 1970 O/L	Greenhouse 1969 O/L
0977	P.I. 158838	Runner	0.84[1/]	0.97 ± 0.03	1.11 ± 0.03
0002	Argentine	Spanish	1.33[1/]	1.37 ± 0.06	1.35 ± 0.07
0112	Spanhoma	Spanish	-	1.36 ± 0.04	-
0972	Ga. 186-28	Runner	0.93[1/]	1.41 ± 0.06	1.21 ± 0.07
0958	NC 5	Va. Bunch	1.86[1/]	2.35 ± 0.16	2.43 ± 0.18
0960	Fla. 393	Runner	1.92[1/]	2.76 ± 0.21	3.20 ± 0.41
0963	Newberry (OK.)	Jumbo	2.31[1/]	3.50 ± 0.30	3.76 ± 0.18
1616	Newberry (GA.)	Jumbo	6.06[2/]	2.81 ± 0.18	3.20 ± 0.59
1617	Bleckley	Jumbo	6.15[2/]	2.51 ± 0.14	3.20 ± 0.37
1618	Korean	Jumbo	6.54[2/]	3.30 ± 0.20	4.76 ± 0.78

1/ Reported by Mason and Matlock (42).

2/ Calculated from the data reported by Holley and Hammons (27).

They represent four types of peanuts, i.e., Spanish, Runner, Virginia, and Jumbo. According to available references, their O/L ratios could roughly be grouped into four different levels: P-977 and P-972 were "very low"; P-2 and P-112, "low"; P-958 and P-960, "medium high"; and P-963, P-1616, P-1617, and P-1618, "high". P-112 is a selection which originated from P-2. Both P-963 and P-1616 are assigned the cultivar name Newberry, but P-963 was introduced to the Oklahoma Agricultural Experiment Station several years earlier than P-1616. All of the "high" O/L ratio cultivars used for the present study were obtained from the Coastal Plain Experiment Station at Tifton, Georgia.

The field trial consisted of two replications each at the Stillwater and Perkins Agronomy Research Stations in 1969 and three replications each at the same stations in 1970. The peanuts were grown in a randomized complete block arrangement. Mature peanuts were shelled and bulked. Ten sound mature kernels from each plot were taken at random for the analysis of O/L ratio. In 1969, three cultivars, P-1616, P-1617, and P-1618, harvested from Perkins, had no sound mature kernels available; therefore, no O/L data were available from these three cultivars in that year.

The O/L ratio of the oil was determined by gas-liquid chromatographic separation of the methyl esters of the fatty acids. The peanut samples were prepared by using a sharp scalpel to slice about one-third of the peanut from the end opposite the germ. With the large-seeded cultivars, it was usually best to remove and discard one-fourth of the kernel before slicing a portion for analysis. The chopped portion of the peanut was placed in a 16 x 150 mm test tube and the following reagents were added in order: 4.0 ml dried benzene, 0.2 ml, 2,

2-dimethoxypropane and 0.5 ml of cold methanolic-HCl. The mixture was shaken and the tubes were covered with cheese cloth and placed on a warm plate overnight to form the methyl esters. Methyl esters of the standard oil samples were also prepared as above for each run. These standards were used to adjust the equipment to obtain accurate analyses. The Barber-Coleman model 5000 gas chromatograph was used for all the studies. The GLC settings used were: detector temperature, 325° C; injector temperature, 245° C; oven temperature, 226°-245° C; and helium flow rate about 200 ml per minute. The retention time of the last peak was approximately three minutes. By this method, only three peaks, palmitic, oleic, and linoleic acids, could be detected. The condition of the GLC was adjusted by using the standard to get an O/L ratio of a range between 1.13 and 1.17. The O/L ratio was determined by the peak height of oleic acid over the peak height of linoleic acid. No correction factor was employed in any test in the present studies.

The analysis of variance was done first on the individual experiments. Information on the importance of cultivar x environment interaction effects was obtained by performing analysis of variance on the combined data. Since no sound mature kernels were available from P-1616, P-1617, and P-1618 in Perkins, 1969, and since each test had two replications in 1969 and three replications in 1970, the data were combined for computing the analyses of variance as follows: 1969 + 1970 at Stillwater, ten cultivars were combined; 1969 + 1970 at Perkins, only seven cultivars (excluding P-1616, P-1617, and P-1618) were combined; Stillwater + Perkins in 1969, seven cultivars and two replications were combined, but ten cultivars and three replications were combined in 1970; Stillwater and Perkins in 1969 and 1970, only seven cultivars

were combined. The statistical procedures reported by Steel and Torrie (54), LeClerg, Leonard and Clark (40), and Comstock and Moll (9) were followed in the present study. It was assumed that years and locations for the present experiment were chosen at random and the ten cultivars were selected as a fixed sample.

II. Results and Discussion

The O/L ratios for each plot are presented in Table II. Each value is the average of ten seeds. For the purpose of illustration, the 1970 experiment conducted at the Stillwater Agronomy Research Station is shown as a frequency histogram in Fig. 1. The ranges of relatively "low" O/L ratio cultivars like P-2, P-112, P-977, and P-972, were narrower than those of relatively "high" ones like P-958, P-960, P-963, P-1616, P-1617, and P-1618, under Stillwater environmental conditions. The mean O/L ratios of each cultivar from this study at two locations for two years are plotted in Fig. 2. It indicates that the mean O/L ratios of the four experiments were similar to one another for each of these five cultivars, P-2, P-977, P-972, P-963, and P-112. These cultivars, except P-963, had a lower O/L ratio relatively. The mean O/L ratios of each of the other cultivars, P-960, P-958 (averaged over four tests), P-1616, P-1617, and P-1618 (averaged over three tests), were relatively higher and showed a wider range in distribution.

All analyses of variance for the individual experiments shown in Table III indicate that the variances of cultivars were highly significantly different from error variances giving evidence of varietal differentiation for O/L ratios. The mean squares for the analyses of variance of combined O/L ratio data are given in Table IV. They

TABLE II

MEAN O/L RATIOS OF TEN CULTIVARS OF PEANUTS FROM EXPERIMENTS CONDUCTED AT TWO LOCATIONS AND FOR TWO YEARS

	Replication Number						
	1969 Stillwater			1970 Stillwater			
P-No.	I	II	Mean	I	II	III	Mean
0002	1.36[1/]	1.41	1.39	1.32	1.32	1.46	1.37
0977	0.91	1.00	0.96	1.00	1.02	0.90	0.97
0960	2.21	2.12	2.18	3.92	2.55	2.43	2.97
0958	1.63	1.59	1.61	2.69	2.39	1.99	2.36
0972	1.13	1.16	1.15	1.43	1.39	1.42	1.41
0963	3.62	3.26	3.44	3.73	3.39	3.36	3.49
1616	3.31	3.60	3.45	3.12	2.67	2.52	2.77
1617	3.02	3.39	3.21	2.56	2.52	2.41	2.50
1618	3.35	3.41	3.38	3.25	3.45	3.21	3.30
0112	1.18	1.34	1.26	1.43	1.34	1.33	1.36
	1969 Perkins			1970 Perkins			
0002	1.29	1.35	1.32	1.39	1.53	1.46	1.46
0977	1.06	0.91	0.99	1.06	1.03	1.16	1.08
0960	2.30	2.10	2.20	2.87	3.15	2.83	2.95
0958	1.93	1.97	1.95	2.18	1.85	2.32	2.12
0972	1.13	1.17	1.15	1.30	1.47	1.43	1.40
0963	3.12	3.36	3.24	3.35	3.14	3.48	3.32
1616	-	-	-	3.27	3.51	4.07	3.62
1617	-	-	-	3.15	3.82	4.24	3.74
1618	-	-	-	3.99	4.37	4.55	4.30
0112	1.30	1.30	1.30	1.54	1.63	1.55	1.57

1/ Each value is the average of 10 determinations.

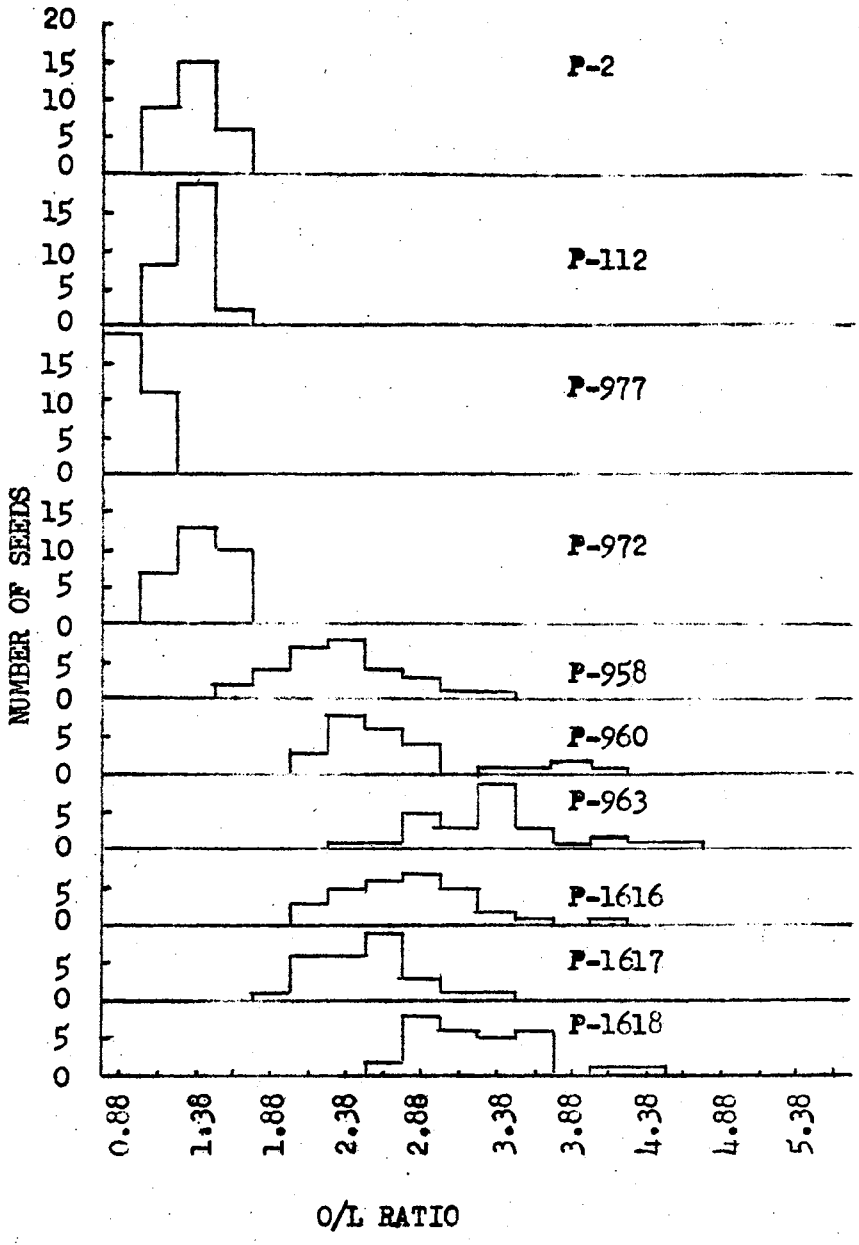

Figure 1. Frequency Histogram of the O/L Ratios of Ten Cultivars from Genotype x Environment Interaction Study Conducted at Stillwater, 1970

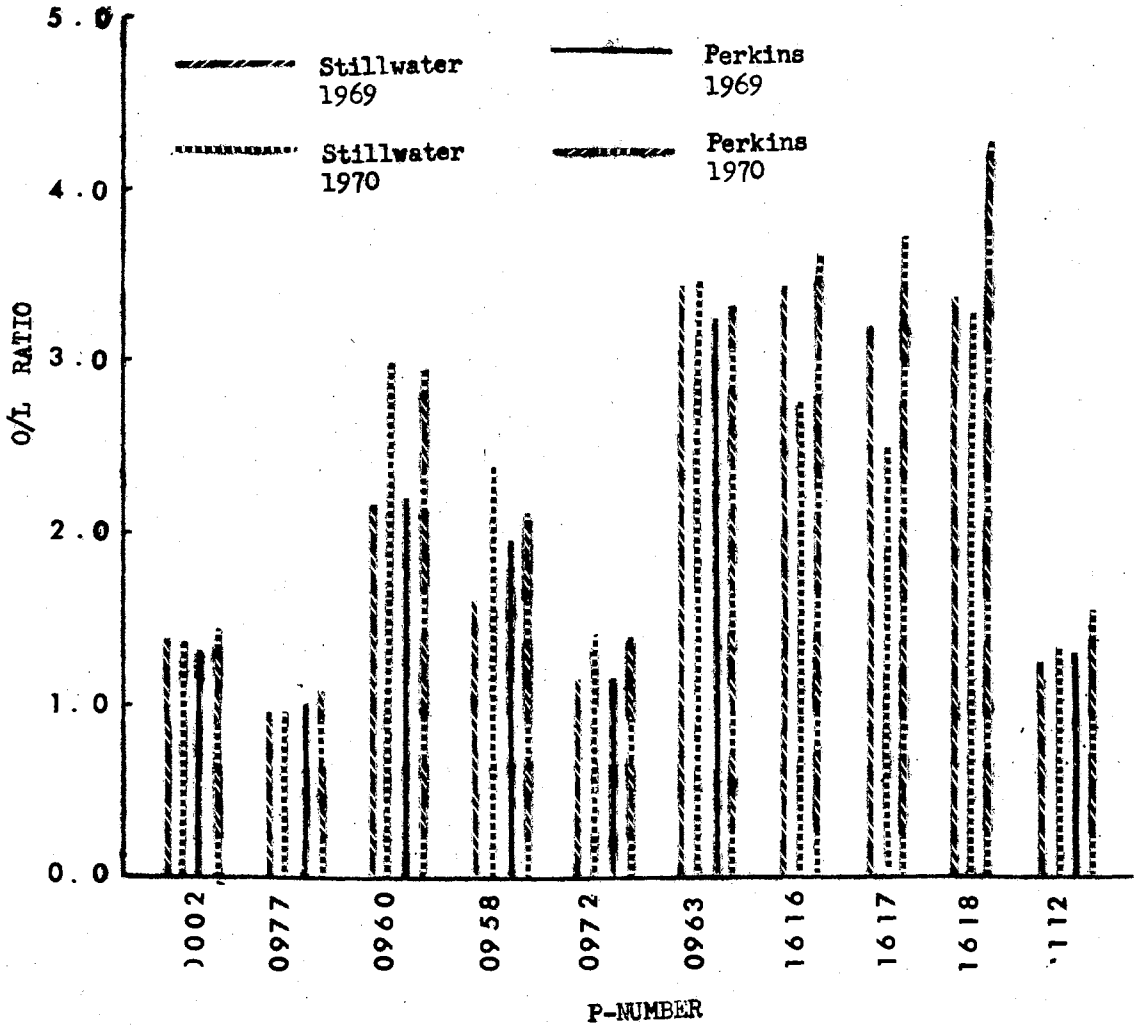

Figure 2. Mean O/L Ratios of Each Cultivar from Genotype x Environment Interaction Study Conducted at Stillwater and Perkins in 1969 and 1970

TABLE III

ANALYSES OF VARIANCE FOR INDIVIDUAL EXPERIMENTS
AT TWO LOCATIONS FOR TWO YEARS

Source	Stillwater				Perkins			
	1969		1970		1969		1970	
	df	Mean Squares	df	Mean Squares	df	Mean Squares	df	Mean Squares
Rep	1	0.0140	2	0.3087	1	0.0001	2	0.2284
Cultivars	9	2.2388**	9	2.4674**	6	1.2646**	9	4.0683**
Rep x Cult.	9	0.0211	18	0.0751	6	0.0106	18	0.0530
Total	19	-	29	-	13	-	29	-

**Significantly different at the .01 level of probability.

TABLE IV

MEAN SQUARES FROM VARIOUSLY COMBINED ANALYSES OF VARIANCE OF 7 OR 10 CULTIVARS GROWN AT TWO LOCATIONS FOR TWO YEARS. (L_1 = STILLWATER: L_2 = PERKINS)

Source	Mean Squares				
	1969 1/ L_1+L_2	1970 L_1+L_2	L_1 1969 + 1970	L_2 1/ 1969 + 1970	L_1+L_2 1/ 1969 + 1970
Locations	0.0041	1.4107	-	-	0.0009
Years	-	-	0.0290	0.5320	1.1883
Loc X Year	-	-	-	-	0.0034
Cultivars	2.7118**	6.1207**	4.4107**	3.3705**	7.4225**
Loc. X Cult.	0.0264^{NS}	0.4149**	-	-	0.0248^{NS}
Year X Cult.	-	-	0.2955**	0.0641*	0.1675**
Loc. X Year X Cult.	-	-	-	-	0.0393^{NS}
Error A	0.0127	0.0641	0.0571	0.0176	0.0969
Error B	-	-	-	-	0.0439

1/ Three varieties, P-1616, P-1617, and P-1618, were excluded

* Significantly different at the .05 level of probability

** Significantly different at the .01 level of probability

NS Not significant

indicate that both cultivars and cultivar x year interaction were highly significant. The second-order interaction, cultivar x year x location, was not significant. The cultivar x location interaction was statistically significant in 1970 but nonsignificant in 1969. It is concluded that cultivars and locations acted independently of each other in 1969, but not in 1970. The analyses also indicate that cultivars and years did not act independently of each other at both locations, Stillwater and Perkins, over 1969 and 1970. The O/L ratios from Stillwater and Perkins in 1969, which resulted in a nonsignificant cultivar x location interaction, were very close to each other in each of the seven cultivars except P-958 as was shown in Fig. 2. P-958 showed a higher O/L ratio in Perkins than in Stillwater.

Fig. 3 illustrates graphically the differences in both direction and magnitude of response of the O/L ratios for seven cultivars from year to year on a location basis and from location to location on a year basis. The responses of these seven cultivars were different. The mean O/L ratios of P-960, P-958, and P-972 showed a considerable increase from 1969 to 1970 at Stillwater but those for P-2, P-963, P-112, and P-977 remained fairly stable over the two years. In Perkins, P-960, P-972, P-958, and P-112 showed larger increases in the mean O/L ratio from 1969 to 1970 than did P-963, P-2, and P-977. Comparisons of the two locations in each of the two years indicated that some of the cultivars responded relatively the same at either location while other cultivars responded slightly different at the two locations. These data also showed that neither location was best for all cultivars, although actual differences were small.

The combined data for two years over two locations did not show

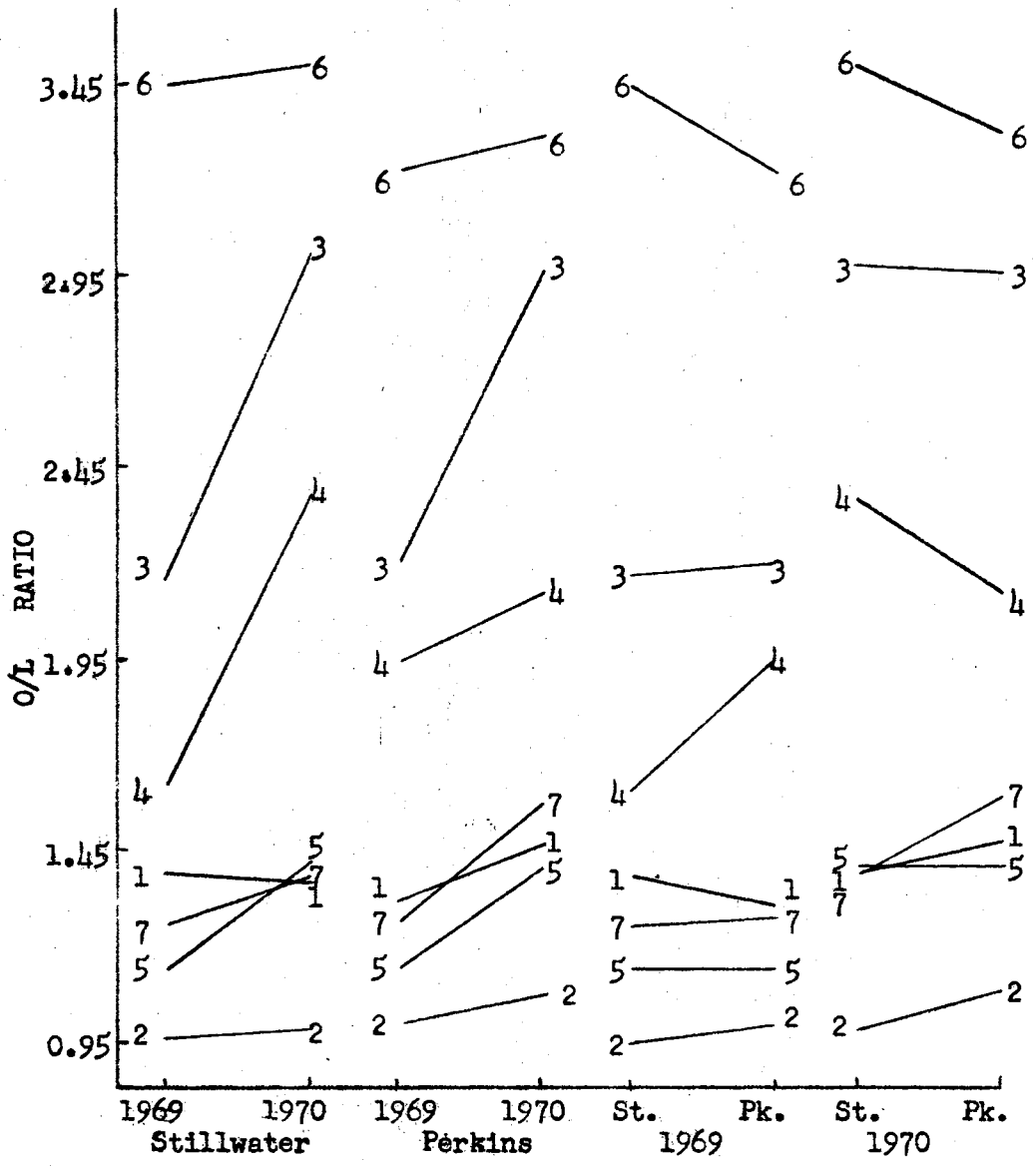

Figure 3. Direction and Magnitude of Response of the O/L Ratios for Seven Cultivars from Year to Year on a Location Basis and from Location to Location on a Year Basis. 1=P-2, 2=P-977, 3=P-960, 4=P-958, 5=P-972, 6=P-963, and 7=P-112

a significant cultivar x location interaction. The very small and nonsignificant cultivar x location interaction indicated that there were consistent location effects on differential varietal response. The locations chosen for the present study, however, are only ten miles apart. The rainfall at Perkins and Stillwater were very much alike as shown in Fig. 4, but Table V shows that the soil properties are very much different. Temperature data were available only from the Stillwater station, however, they would presumably be very much similar to each other at the same period of time.

Table VI indicates differences in O/L ratios among the ten selected cultivars of peanuts. Duncan's new multiple range test (54) was used to compare the differences among the mean O/L ratios. Essentially, these results supported the earlier "four-group" classification. P-977 appeared to be the lowest cultivar in O/L ratio in all four tests. P-972 had a higher ratio in the present studies than what had been reported earlier (42), even though it did not differ significantly from P-977 except in the combined analysis of two locations in 1969. P-2 and P-112 exhibited O/L ratios intermediate to the "low" and "medium" O/L ratio groups. P-112 is a selection from P-2. The O/L ratio of these two cultivars did not differ significantly, but, in most cases, P-112 had a slightly lower O/L ratio than P-2.

In an earlier classification, P-960 and P-958 were classified in the same group. The present results support their classification as "medium high" in this group of ten cultivars, however, P-960 consistently had a higher O/L ratio than P-958 and the difference was statistically significant in all but one analysis. The present studies support the classification of the four "high" O/L cultivars, P-963,

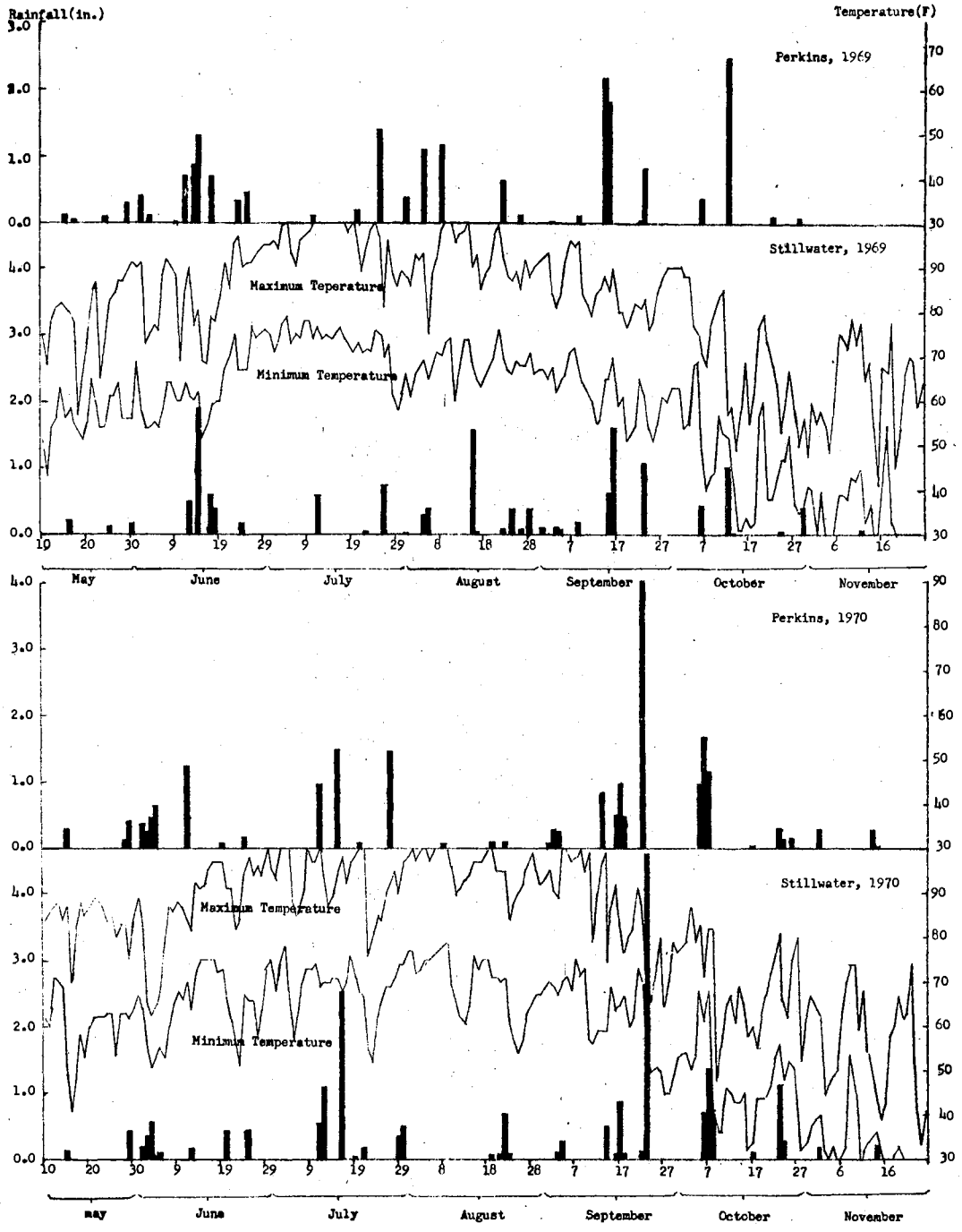

Figure 4. Daily Precipitation and Temperature at Stillwater Agronomy Research Station and Daily Precipitation at Perkins from May 10 to November 25, 1969-1970

TABLE V

SOIL PROPERTIES OF TEST PLOTS FROM GENOTYPE X ENVIRONMENT INTERACTION STUDY AT STILLWATER AND PERKINS AGRONOMY RESEARCH STATIONS 1/

Location	Soil Reaction (pH)	Organic Matter %	Organic Matter Level	Phosphorus #/A	Phosphorus Level	Potassium #/A	Potassium Level	Mg #/A	Ca #/A	Soil Type
Perkins	5.0	1.35	Very Low	70	Very High	394	Very High	352	1050	Taller loam
Stillwater	6.5	0.65	Very Low	42	Medium High	330	Very High	660	1473	Yahola Fine Sandy Loam

1/ Soil test was conducted by the Soil and Water Service Laboratory, Department of Agronomy, Oklahoma State University

TABLE VI

MULTIPLE RANGE TESTS OF THE MEAN O/L RATIOS OF INDIVIDUAL AND COMBINED EXPERIMENTS FROM THE GENOTYPE X ENVIRONMENT INTERACTION STUDY OF TEN PEANUT CULTIVARS GROWN AT TWO LOCATIONS FOR TWO YEARS

	Mean Oleic-Linoleic Acid Ratio				
P-No.	1969 Stillwater	1970 Stillwater	1969 1/ Perkins	1970 Perkins	1969 2/ Stillwater + Perkins
0977	0.95 a 3/	0.97 a	0.99 a	1.08 a	0.97 a
0972	1.15 a	1.41 a	1.15 ab	1.40 a	1.15 b
0112	1.26 ab	1.37 a	1.30 b	1.46 ab	1.28 bc
0002	1.39 bc	1.37 a	1.32 b	1.57 b	1.35 c
0958	1.61 c	2.36 b	1.95 c	2.12 c	1.78 d
0960	2.18 d	2.97 cd	2.20 cd	2.95 d	2.19 e
0963	3.44 e	3.49 f	3.24 d	3.32 de	3.34 f
1616	3.55 e	2.77 bc	-	3.62 ef	-
1617	3.21 e	2.50 b	-	3.75 f	-
1618	3.38 e	3.30 ef	-	4.30 g	-

1/ No sound mature kernels were available for O/L ratio analysis from P-1616, P-1617, and P-1618.

2/ P-1616, P-1617, and P-1618 were omitted from these combined data.

3/ Means followed by the same letter are not significantly different at the .05 level of probability.

P-1616, P-1617, and P-1618. However, these cultivars showed more variability between individual seeds for O/L ratios than did the lower O/L groups. Whether the "high" O/L ratio is more sensitive to environmental changes than "low" or "medium" levels is not answered in the present study.

The only combined analysis that did not show significant interaction was the 1969 data combined over the two locations. The combined mean O/L ratios were compared using Duncan's new multiple range test. Three of the "high" O/L cultivars were omitted from this analysis but the mean separation again supports the overall classification of each cultivar.

In all analyses of variance, the mean squares due to cultivars were larger than any other source; therefore, the genotype contribution to the O/L ratio may be considered more important. Since these cultivars were chosen based on their previously determined O/L ratio information, their differences were expected and confirmed by the present study. Genotype x year interaction, but not genotype x location, was consistently statistically significant as shown by the combined analyses. These results indicate that the cultivars responded in different ways in the two years. The second-order interaction of cultivar x year x location was very small and nonsignificant.

CHAPTER IV

MATERNAL INFLUENCE ON O/L RATIO OF PEANUTS

Each peanut seed is composed of two massive seed leaves (cotyledons) and one germ which all originate from the fertilized egg. Techniques for rapid analysis of fatty acid composition of peanut seed are now available (63). These techniques do not destroy the seeds, permitting genetic and breeding selection studies on oil quality to be made on single seeds. The presence or absence of maternal effects on oil quality must be established to determine the generations for selection.

The purpose of the present study was to determine whether the O/L ratio of peanut seeds depends upon the genotype of the seed, the genotype of the maternal parent, or a combination of both. Reciprocal crosses and backcrosses were made. Since reciprocal crosses are genetically similar, any differences in O/L ratio can be attributed to maternal influence.

I. Materials and Methods

The same peanut accessions used in the genotype x environment interaction study were used for the present study with the exception of two cultivars, P-112 and P-958. To assure information on the O/L ratios of cultivars to be used in a hybridization program, five sound

mature kernels of each accession were carefully selected and sent to the Biochemistry Laboratory, Oklahoma State University, for O/L analysis. The rapid O/L procedure and half-seed techniques were used as described in Chapter III. The embryo portions of the seeds were planted in the greenhouse in the early spring of 1969. Two plants from each cultivar were selected for making handcrosses to obtain F_1 seeds. All reciprocal crosses were attempted to achieve the purpose of the present study. The maternal and paternal parents of a cross are designated throughout by the first and second digits of the pair, respectively. As soon as the crossed fruits were mature, they were harvested and dried. After shelling, sound mature F_1 seeds were sent to the Biochemistry Laboratory for O/L determination. A total of 75 F_1 half-seeds were selected and planted in the same greenhouse in the early spring of 1970 to develop F_2 seeds. Several cuttings from F_1 plants of crosses P-2 x P-977, P-2 x P-963, and P-2 x P-1618 were made and grown in a growth chamber. They were used as female and/or pollen parents for making as many backcrosses as possible.

Analyses of variance for the O/L ratios from F_1's and backcrosses were carried out by using statistical methods for samples of unequal size (52). Mean O/L ratios of parents, reciprocal crosses, and backcrosses were compared for statistical significance within groups by Kramer's multiple range test (38).

In addition to the data from F_1's and backcrosses, remnant F_2 seeds of two crosses, P-960 x P-2 and P-977 x P-2, in which their F_1 hybrids were made in 1966, were available and were planted at the Perkins Agronomy Research Station to develop F_3 seeds for the present study. They were space-planted in the middle of May, 1969, and were harvested

in late October. After drying and shelling, seven sound mature kernels were selected at random from each F_2 plant for the analysis of the fatty acids. The crosses P-977 x P-2 and P-960 x P-2 were repeated and F_1 seeds were available in 1969. These F_1 seeds were planted at the Stillwater Agronomy Research Station to develop F_2 seeds for the present study.

II. Results and Discussion

The mean O/L ratios of parental cultivars and their hybrids are presented in Table VII. The O/L ratio from the cross 1 x 2, was much closer to its "higher" O/L parent, P-2, which was the paternal parent in the cross. The reciprocal cross, 2 x 1, was not significantly different from either parent. Crosses 1 x 3, 1 x 4, 2 x 4, 2 x 3, 2 x 5, and 6 x 5 did not differ significantly from their respective maternal parent value for the O/L ratio, but they did differ significantly from their respective paternal parents. However, their respective reciprocal crosses, except 3 x 2, were significantly different from their maternal parents. Two crosses, 1 x 5 and 6 x 7, and their reciprocal crosses, 5 x 1 and 7 x 6, were statistically different from their respective maternal parents. In one cross, 3 x 8, the O/L ratio differed significantly from its maternal parent, but its reciprocal cross, 8 x 3, did not.

If the "high" and "low" O/L values are conditioned by nuclear factors, the F_2 and BC_1 seeds must show segregation or a difference from their maternal parent in the O/L ratio; otherwise, this character must be controlled by the genetic constitution of the maternal parent which contributes nuclear and cytoplasmic effects.

TABLE VII

MEAN O/L RATIOS OF PARENTS AND CROSSES

Parents or Crosses	No. of Seeds	Mean O/L		Standard Error
P-977 (1)	17	1.10	a[1]	0.01
1 x 2 [2]	1	1.29[3]		-
2 x 1	11	1.02	a	0.02
P-2(2)	10	1.35	abcde	0.03
P-977 (1)	17	1.10	a	0.01
1 x 3	2	1.12	abcde	0.02
3 x 1	11	1.72	abcdef	0.06
P-960 (3)	13	3.19	gh	0.18
P-977 (1)	17	1.10	a	0.01
1 x 4	7	1.44	abcde	0.14
4 x 1	3	1.49	abcde	0.10
P-963 (4)	9	3.72	hi	0.19
P-977 (1)	17	1.10	a	0.01
1 x 5	15	2.15	cdefg	0.19
5 x 1	11	1.54	abcde	0.10
P-1618(5)	20	4.73	j	0.37
P-972 (6)	10	1.20	abcde	0.03
6 x 7	5	2.82	fg	0.12
7 x 6	8	1.72	abcdef	0.13
P-1616(7)	9	3.20	gh	0.25
P-972 (6)	10	1.20	abcde	0.03
6 x 5	4	2.33	defg	0.19
5 x 6	2	1.71	abcdef	0.17
P-1618(5)	20	4.73	j	0.37
P-2 (2)	10	1.35	abcde	0.03
2 x 4	7	1.89	abcdef	0.13
4 x 2	5	1.98	abcdef	0.16
P-963 (4)	9	3.72	hi	0.19
P-2 (2)	10	1.35	abcde	0.03
2 x 5	11	2.08	bcdefg	0.06
5 x 2	2	2.03	abcdefg	0.34
P-1618 (5)	20	4.73	j	0.37
P-2 (2)	10	1.35	abcde	0.03
2 x 3	5	1.31	abcde	0.06
3 x 2	3	2.50	efg	0.46
P-960 (3)	13	3.19	gh	0.18
P-960 (3)	13	3.19	gh	0.18
3 x 8	17	4.15	i	0.24
8 x 3	6	3.11	gh	0.12
P-1617 (8)	20	3.20	gh	0.17

1/ Means followed by the same letter are not significantly different at .05 level of probability
2/ Maternal parent listed first in each cross
3/ Excluded from this comparison

Three F_1 plants, P-2 x P-977, P-2 x P-963, and P-2 x P-1618, were used as female and/or pollen parents for making backcrosses with one or both of their parents. The results are given in Table VIII. Two backcrosses, P-2 x F_1 (P-2 x P-977) and P-977 x F_1 (P-2 x P-977), were not significantly different from their respective paternal and maternal values for the O/L ratio. The parents involved in these two backcrosses likewise did not differ. In the backcross, F_1 (P-2 x P-963) x P-2, the O/L ratio did not significantly differ from its maternal parent, but differed significantly from its paternal parent. Two backcrosses, P-2 x F_1 (P-2 x P-963) and P-2 x F_1 (P-2 x P-1618), were not significantly different from either their maternal or their paternal value for the O/L ratio. Two backcrosses, F_1 (P-2 x P-1618) x P-2 and P-1618 x F_1 (P-2 x P-1618), and a three-way cross, F_1 (P-2 x P-1618) x P-977, were significantly different from their respective maternal values for the O/L ratio, but they did not differ significantly from their respective paternal parents.

Both F_1 and BC_1 data lacked strong and consistent evidence indicating maternal influence on the O/L ratio in peanuts. It is, nevertheless, realized that sample numbers were relatively small and limited. Theoretically, due to maternal influence the nuclear effect might not be detected from the results of the F_1 and BC_1 seeds. It should be, therefore, greatly helpful to have data from F_2 and F_3 generations to support this conclusion.

Frequency histograms of the O/L ratio from F_2 and F_3 seeds and F_2 plant means are shown in Figs. 5 through 8. The O/L ratio distribution of the F_2 plant means from the cross, P-977 x P-2, is very similar to that of the F_2 seeds. Both of them show a fairly normal distribution.

Table VIII

MEAN O/L RATIOS OF PARENTS AND BACKCROSSES

Parent or Backcross	No. of Seeds	Mean O/L	Standard Error
P-2 (1)	10	1.35 abcde[1/]	0.03
1 x 2	17	1.30 abc	0.04
F_1(P-2 x P-977) (2)	11	1.02 a	0.02
P-977 (3)	17	1.10 a	0.01
3 x 2	8	1.16 abc	0.07
F_1(P-2 x P-977) (2)	11	1.02 a	0.02
F_1(P-2 x P-963) (4)	7	1.89 efg	0.13
4 x 1	31	1.80 defg	0.06
P-2 (1)	10	1.35 a	0.03
P-2 (1)	10	1.35 abcde	0.03
1 x 4	8	1.43 abcde	0.08
F_1(P-2 x P-963) (4)	7	1.89 efg	0.13
P-2 (1)	10	1.35 abcde	0.03
1 x 5	12	1.64 cdef	0.11
F_1(P-2 x P-1618) (5)	11	2.08 fg	0.07
F_1(P-2 x P-1618) (5)	11	2.08 fg	0.07
5 x 1	35	1.58 bcde	0.05
P-2 (1)	10	1.35 abcde	0.03
P-1616 (6)	20	4.73 h	0.38
6 x 5	15	2.18 g	0.13
F_1(P-2 x P-1618) (5)	11	2.08 fg	0.07
F_1(P-2 x P-1618) (5)	11	2.08 fg	0.07
5 x 3*	15	1.03 a	0.04
P-977 (3)	17	1.10 a	0.01

1/ Means followed by the same letter are not significantly different at the .05 level of probability.

* A three-way cross.

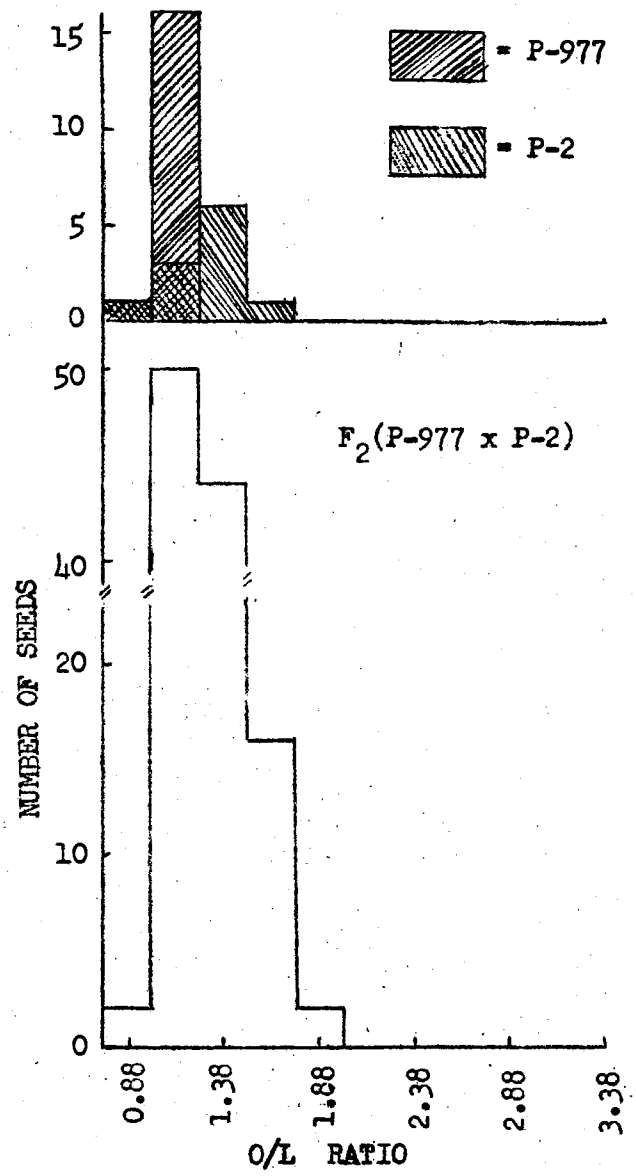

Figure 5. Frequency Histogram of O/L Ratios from F_2 Seeds of Cross P-977 x P-2 and Their Parents Grown in the Field, Stillwater, 1970

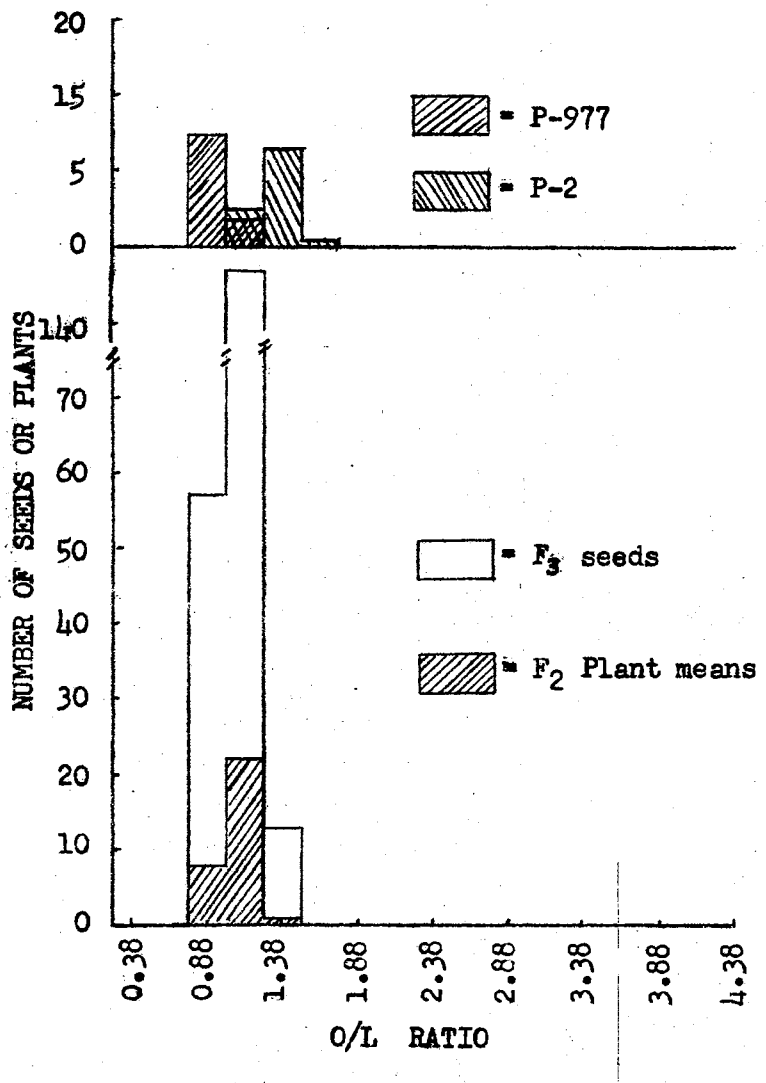

Figure 6. Frequency Histogram of O/L Ratios of F_3 Seeds, F_2 Plant Means, and Parents of Cross P-977 x P-2 Grown in the Field, Perkins, 1969

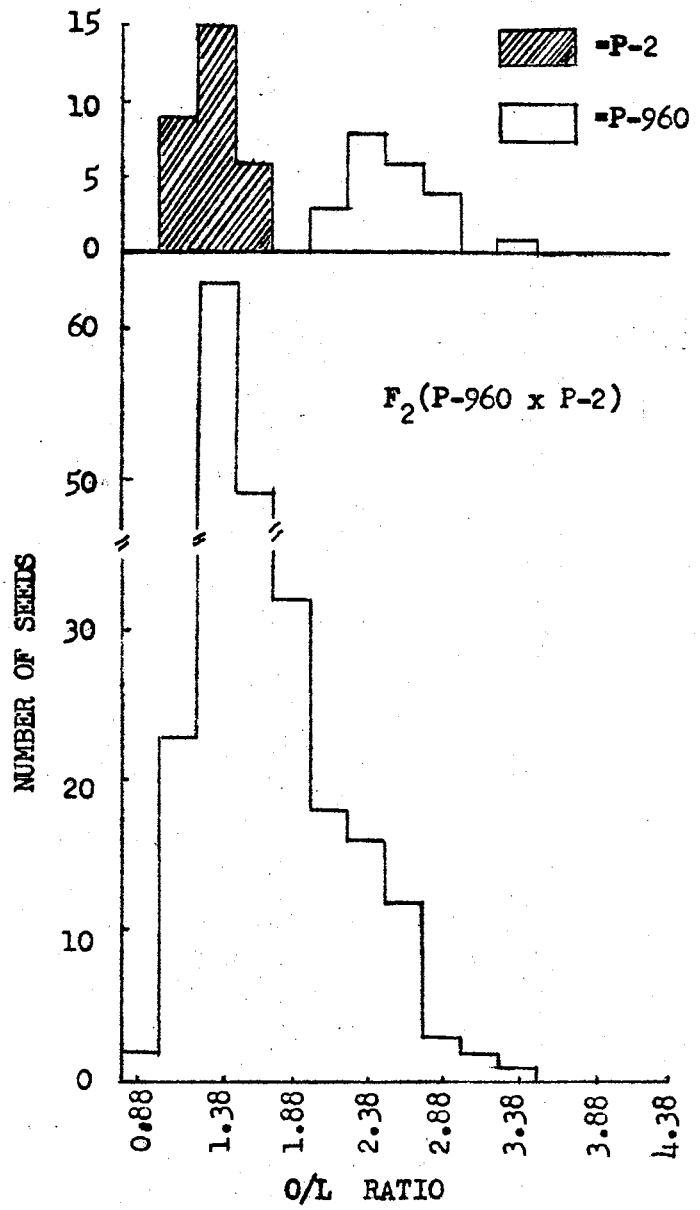

Figure 7. Frequency Histogram of O/L Ratios of F_2 and Parents of Cross P-960 x P-2 Grown in the Field, Stillwater, 1970

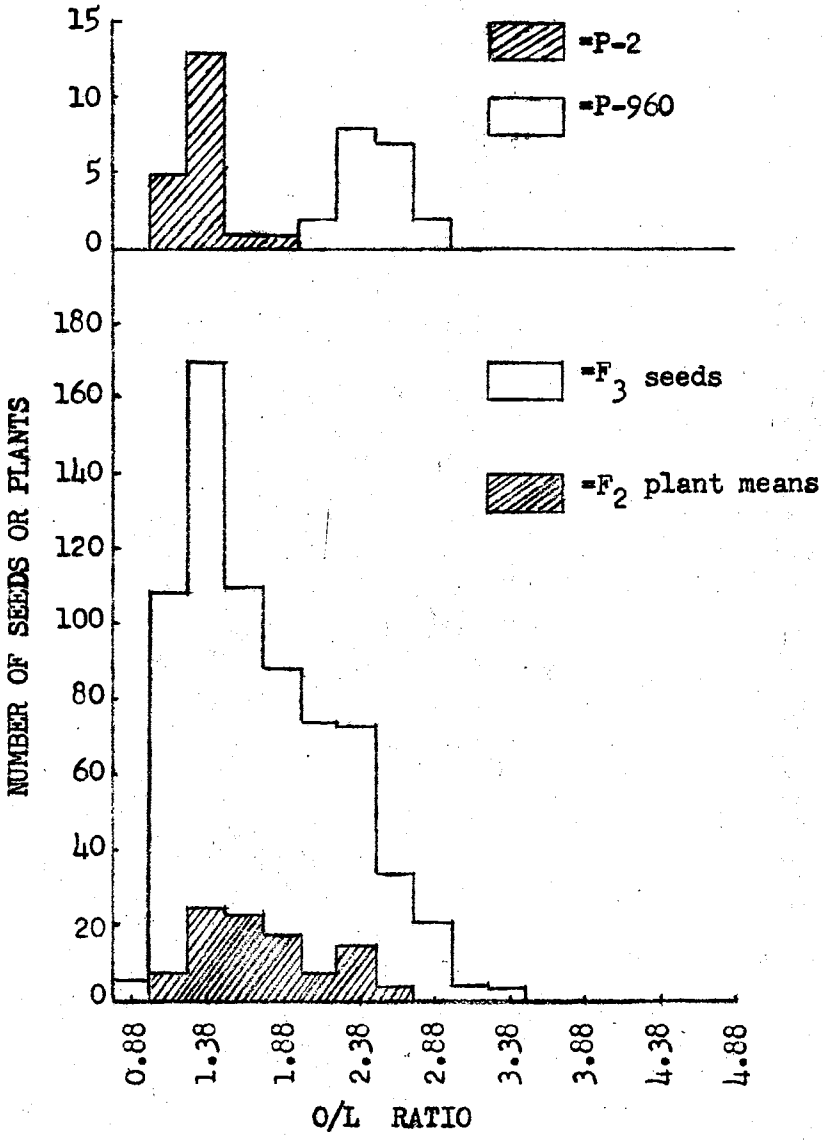

Figure 8. Frequency Histogram of O/L Ratios of F_3 Seeds, F_2 Plant Means, and Parents of Cross P-960 x P-2 Grown in the Field, Perkins, 1969.

So did the F_3 seeds from the same cross. Each of these populations had a mean approximately intermediate between the two parents. In the cross, P-960 x P-2, the frequency distribution of the O/L ratio of F_3 seeds is very similar to that of F_2 seeds. Both are skewed toward the "low" O/L ratio parent, P-2. The frequency distributions for both F_2 seeds and F_2 plant means show continuous variation. These results indicate that all the seeds from F_1 plants did not have the same value for the O/L ratio as would be expected under strict maternal effects. Further evidence is provided that the O/L ratio in peanuts must be controlled by the genetic constitution of the seed and not by that of the maternal plant.

CHAPTER V

INHERITANCE OF O/L RATIO

The only known report concerning O/L ratio inheritance in peanuts was published by Mason and Matlock (42). Based upon limited data of bulk samples from natural hybridization materials, they concluded that the ratio of oleic to linoleic acid was quantitatively inherited and controlled by multiple factors. Since then, the half-seed technique and rapid O/L ratio procedures have become available. The purpose of the present study was to determine the mode of inheritance of the O/L ratio in peanuts by using this new technique and procedure. Data from F_1, backcross, F_2 and F_3 generations were obtained from controlled crosses and are presented.

I. Materials and Methods

The same materials used in the study of the maternal effects presented in Chapter IV were used for the present study. The following thirteen crosses were selected for intensive study:

P-2 x P-972	P-977 x P-2
P-2 x P-977	P-977 x P-960
P-2 x P-960	P-977 x P-1618
P-2 x P-963	P-1616 x P-977
P-2 x P-1618	P-1616 x P-972
P-960 x P-2	P-1617 x P-960
P-960 x P-977	

All of these F_1 hybrids, except P-977 x P-2, P-960 x P-977, and P-960 x P-2 which were grown at the Stillwater Agronomy Research

Station, were planted in the greenhouse in early Spring, 1970, and were harvested in October in the same year. The F_2 seeds were dried between 18 and 35° C for one week. After shelling, they were kept in cold storage at a temperature of 7° C until the fatty acid analysis was made. The same procedure as described in Chapter III was used. All sound mature F_2 seeds were selected from each population for the analysis of the fatty acids for this study.

Backcross populations mentioned in Chapter IV were also available for this portion of the study.

Remnant F_2 seeds of two crosses, P-960 x P-2 and P-977 x P-2, in which the F_1 hybrids were made in 1966, were also used in this study.

II. Results and Discussion

The parental cultivars used fell into approximately four groups in regard to the O/L ratio. This has been discussed in detail in Chapter III (Tables I and VI, and Fig. 1). P-977 was "very low" in O/L ratio while P-2 and P-972 were classed as "low". P-960 was "medium" in O/L ratio and P-963, P-1616, P-1617, and P-1618 were "high". This classification is not absolute since variation is present as shown in Tables I and VI. In an earlier report (42), the O/L ratio of P-972 was less than 1.00, but the present results show it to be about the same as P-2 and P-112. As mentioned earlier, the seed from "high" O/L ratio cultivars exhibited a wider range of O/L ratios than those from "very low" or "low" cultivars. P-960, with a "medium" O/L ratio, varied like the "high" ones under greenhouse conditions.

Summary data obtained from crosses of parents differing in levels of O/L ratios indicate that in most cases the F_1 seeds were intermediate

between the parents but were closer to the low O/L parent (Table VII). There were exceptions: P-2 x P-977 and P-2 x P-960 had a lower O/L than their "low" parents and P-960 x P-1617 had a higher O/L than its "high" parent.

Mean O/L ratios of the seeds of backcrosses and their parents are given in Table VIII. In most cases, the O/L ratio of BC_1 seeds was intermediate between the parents. Frequency histograms which indicate fairly normal distributions are shown in Figs. 9, 10, and 11.

Mean O/L ratios, ranges, and standard errors of F_2 populations are summarized in Table IX. The F_2 means were approximately intermediate between the parental means which were shown in Table I. In these F_2 populations, the continuous range of variation from low to high values indicates a quantitative inheritance of O/L ratio in peanuts. The two crosses, P-977 x P-1618 and P-1616 x P-977, produced the widest range among the F_2 populations, as expected, since P-977 represented the extreme low O/L ratio and P-1616 and P-1618 represented the extreme high O/L ratio. The cross, P-2 x P-972, showed the narrowest range among the F_2 populations which was also expected, since both parents had approximately the same level of O/L ratio.

The F_2 populations obtained from reciprocal crosses between "very low" and "low" parents, i.e., P-977 x P-2 and P-2 x P-977, showed transgressive segregation to both parents. The F_2 population from P-2 x P-977 had a wider range of O/L ratios than that from P-977 x P-2. The former population also showed less standard error than the latter one. These two populations had fairly normal distributions of the O/L frequency as was shown in Figs. 5 and 9. The frequency distributions from backcrosses to both parents also indicated quantitative

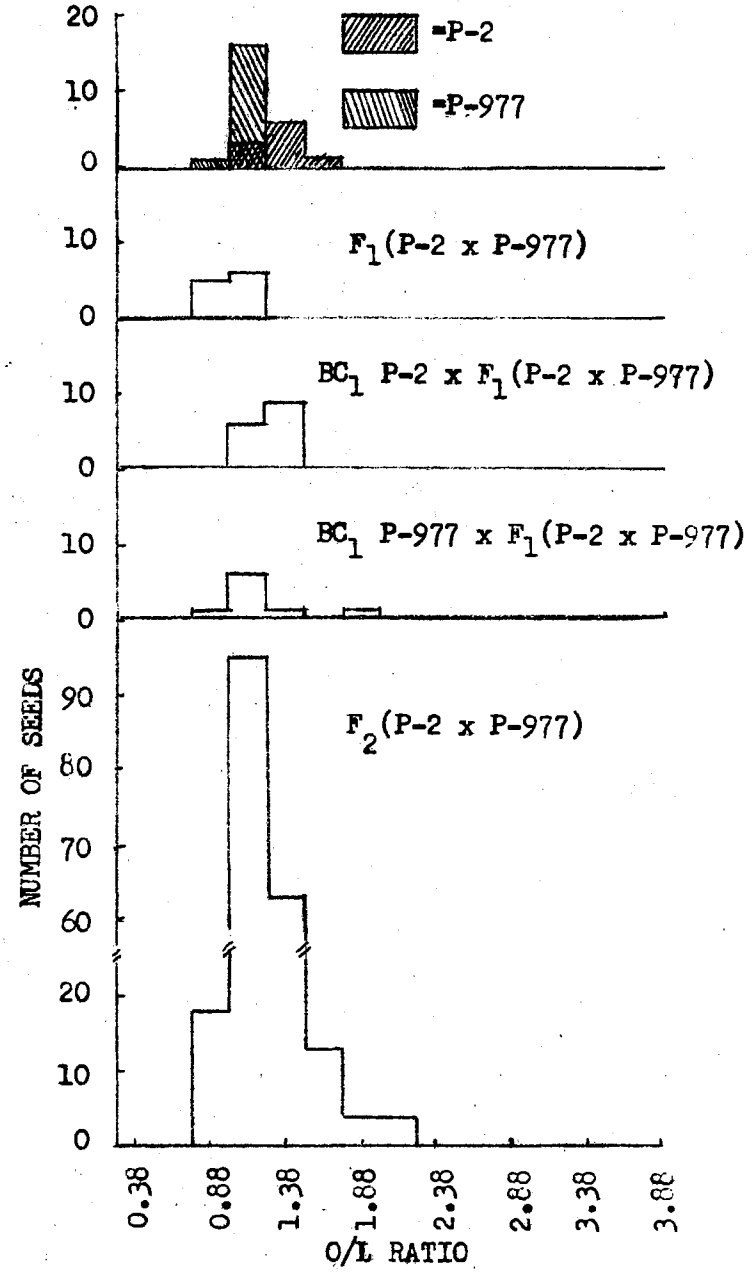

Figure 9. Frequency Histogram of O/L Ratios from F_1, F_2, BC_1, and Parents of Cross P-2 x P-977 Grown in the Greenhouse

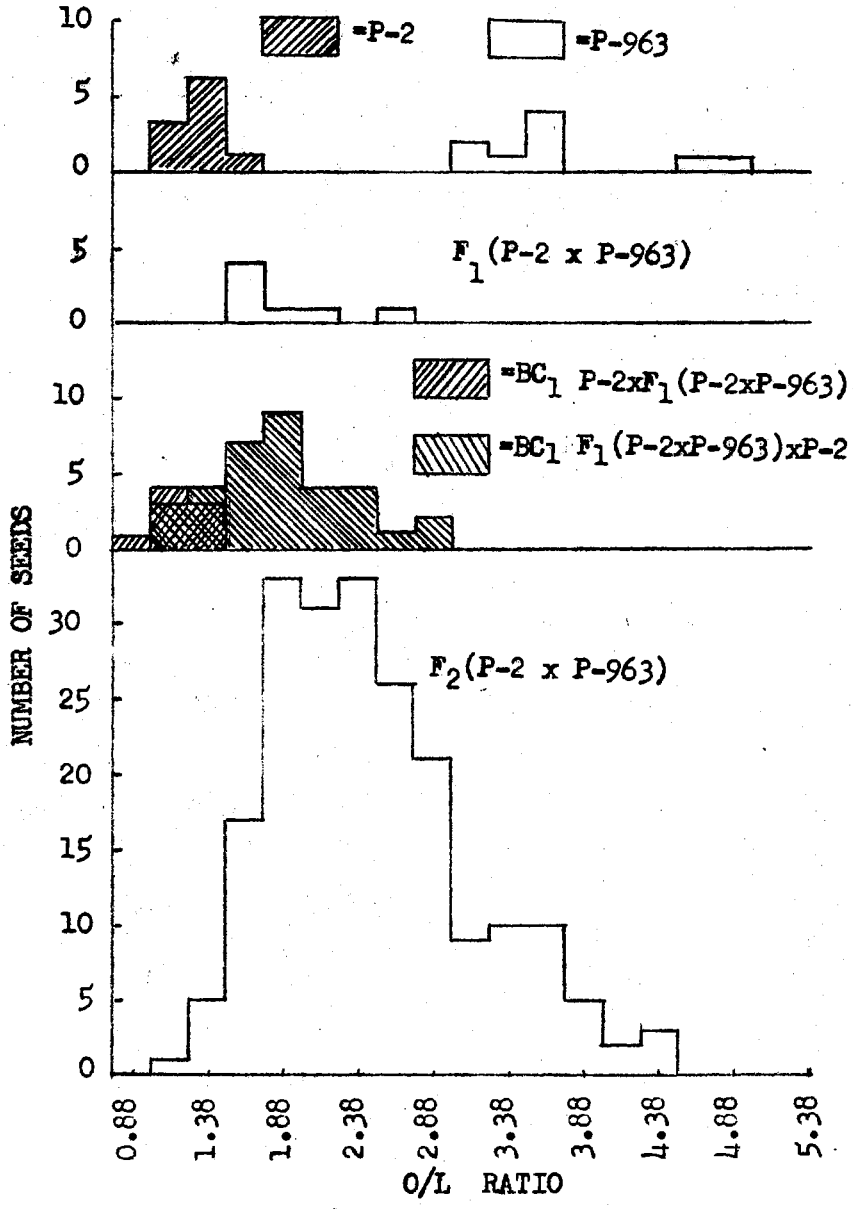

Figure 10. Frequency Histogram of O/L Ratios of F_1, F_2, BC_1, and Parents of Cross P-2 x P-963 Grown in the Greenhouse

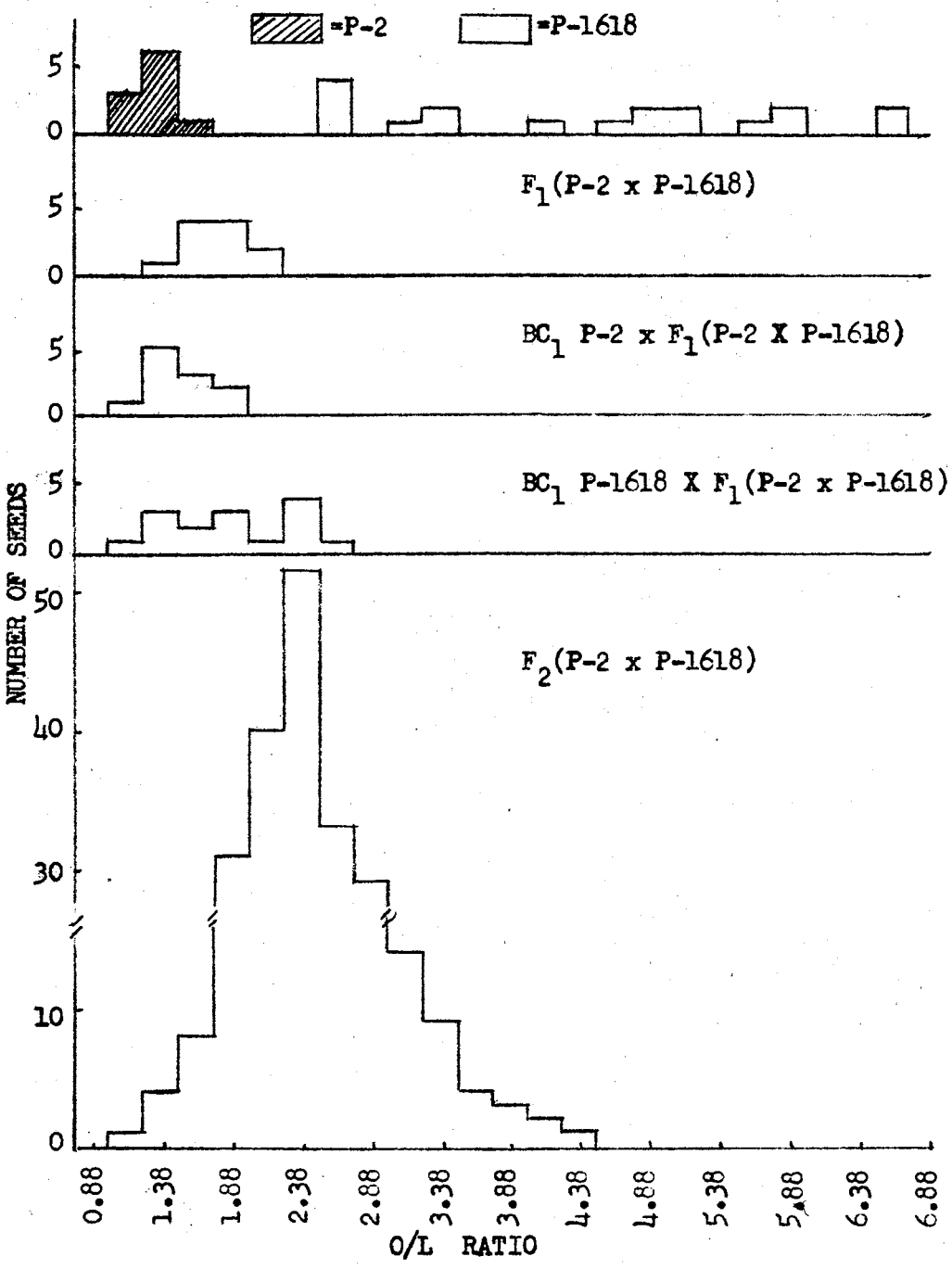

Figure 11. Frequency Histogram of O/L Ratios of F_1, F_2, BC_1, and Parents of Cross P-2 x P-1618 Grown in the Greenhouse

TABLE IX

MEAN O/L RATIOS DETERMINED BY HALF-SEED ANALYSIS FOR F_2 FAMILIES GROWN IN THE GREENHOUSE

Cross	No. of F_2 Seeds	Range	Mean	Standard Error
P-977 X P-2*	115	0.96 - 1.74	1.29	0.02
P-2 X P-977	196	0.86 - 2.18	1.26	0.01
P-977 X P-960*	161	1.03 - 3.50	1.67	0.03
P-960 X P-977*	154	0.93 - 2.84	1.49	0.03
P-977 X P-1618	185	1.03 - 5.22	2.37	0.06
P-1616 X P-977	47	1.02 - 4.91	2.05	0.11
P-2 X P-972	63	1.03 - 1.75	1.40	0.02
P-2 X P-960*	256	1.06 - 4.62	2.27	0.04
P-960 X P-2*	222	0.99 - 3.33	1.73	0.03
P-2 X P-963	207	1.21 - 4.57	2.50	0.05
P-2 X P-1618	238	1.33 - 4.45	2.44	0.04
P-1616 X P-972	45	1.19 - 3.04	1.96	0.06
P-1617 X P-960	133	2.34 - 6.00	4.07	0.09

* These were grown in the field at the Stillwater Agronomy Research Farm, 1970

inheritance.

The frequency distributions of two "very low" x "high" crosses, P-977 x P-1618 and P-1616 x P-977, are shown in Figs. 12 and 13. Both F_2 populations approached a normal distribution, however, both were slightly skewed toward the lower O/L value parent. These two F_2 populations showed the widest ranges of O/L ratios as indicated in Table IX.

One "low" x "low" cross, P-2 x P-972, resulted in an F_2 population with a fairly normal distribution as shown in Fig. 14. However, the range was very narrow and no trangressive segregation was obtained.

In reciprocal crosses, P-977 x P-960 and P-960 x P-977 which were crosses between "very low" and "medium high" parents for the O/L value, the frequency distributions of the F_2 populations were skewed toward the "very low" O/L parent, P-977, as shown in Figs. 15 and 16. Although the F_2 of P-977 x P-960 was grown in the greenhouse and the F_2 of P-960 x P-977 on the Stillwater Agronomy Research Farm, they appeared to have frequency distributions very similar to each other. However, the F_2 population grown in the field exhibited a narrower range of O/L ratios and slightly lower values than the reciprocal F_2 population grown in the greenhouse.

The reciprocal crosses, P-2 x P-960 and P-960 x P-2, represent a cross of "low" x "medium high". F_2 seeds of P-2 x P-960 were harvested from the greenhouse while the reciprocal cross was grown on the Stillwater Agronomy Research Farm in 1970. The frequency distributions of these reciprocal populations matched each other closely and tended toward the "low" O/L parent, P-2, as shown in Figs. 7 and 17. The data from these two reciprocal populations and from the reciprocal F_2

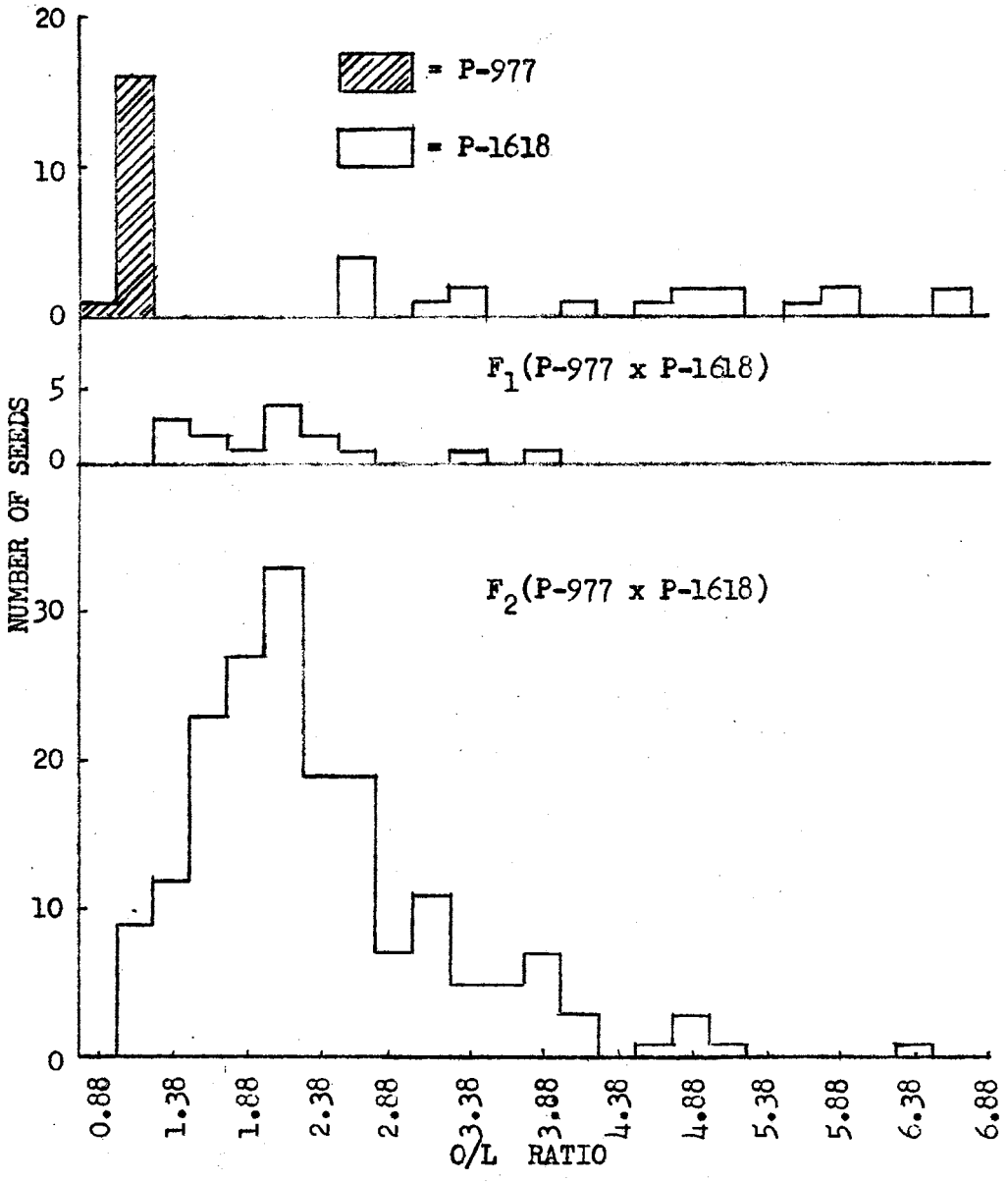

Figure 12. Frequency Histogram of O/L Ratios from F_1, F_2, and Parents of Cross P-977 x P-1618 Grown in the Greenhouse

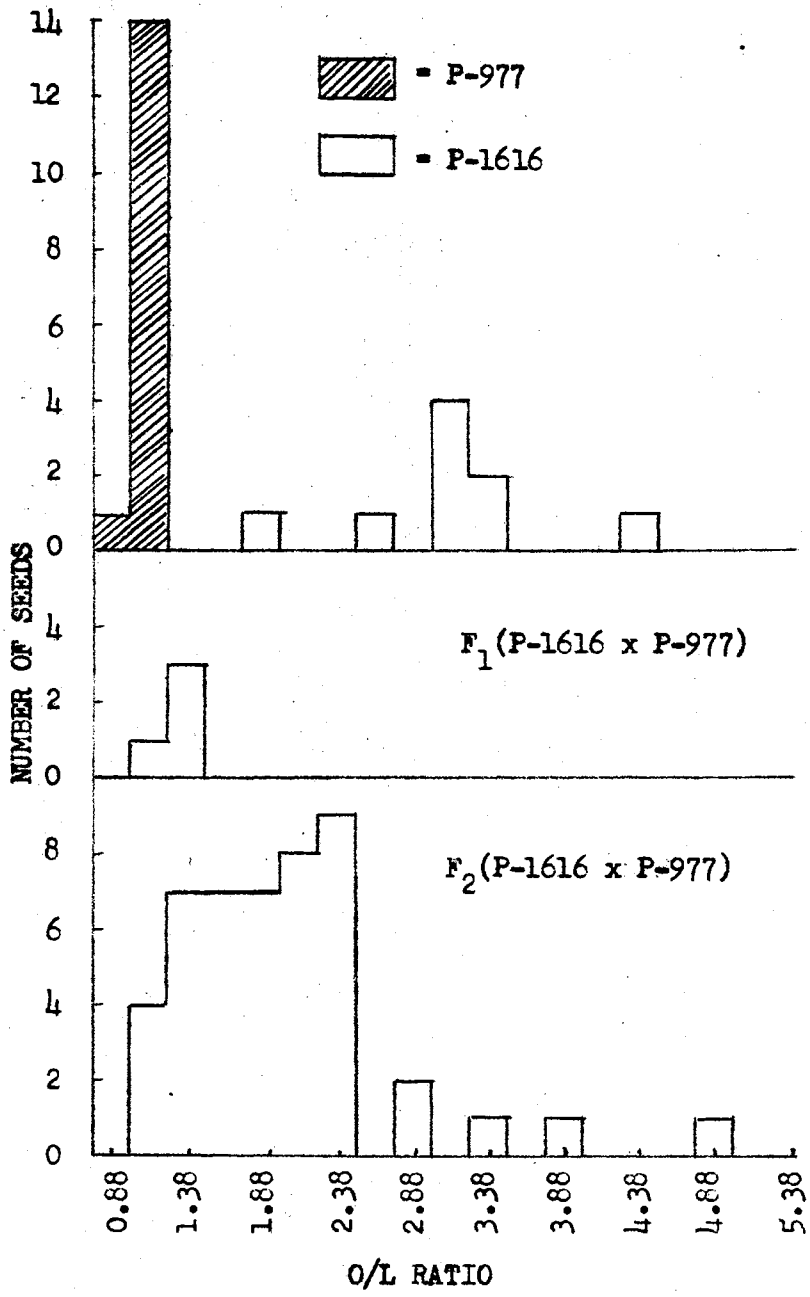

Figure 13. Frequency Histogram of O/L Ratios of F_1, F_2, and Parents of Cross P-1616 x P-977 Grown in the Greenhouse

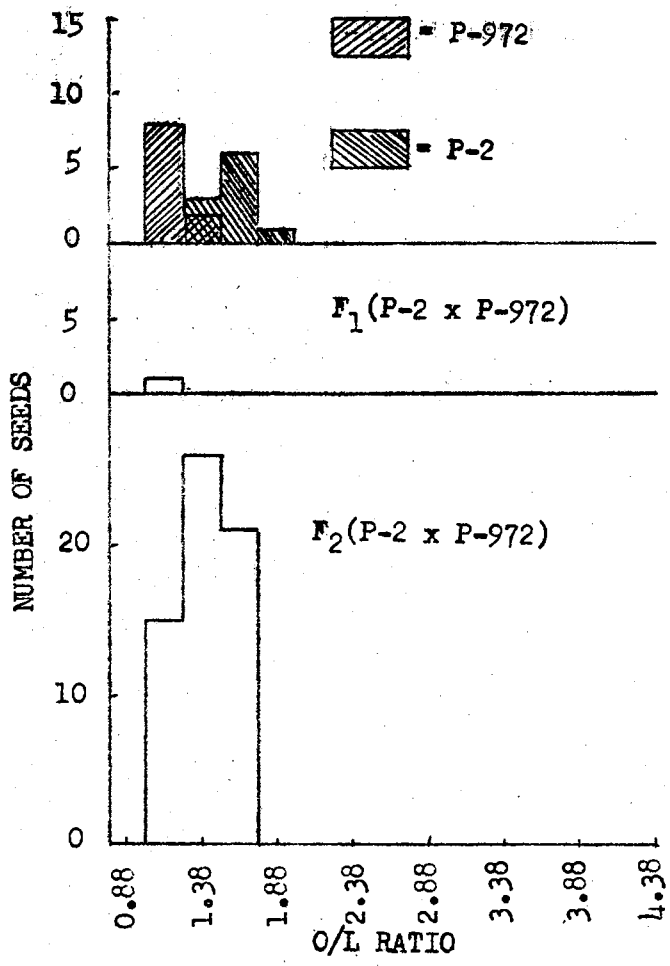

Figure 14. Frequency Histogram of O/L Ratios of F_1, F_2, and Parents of Cross P-2 x P-972 Grown in the Greenhouse

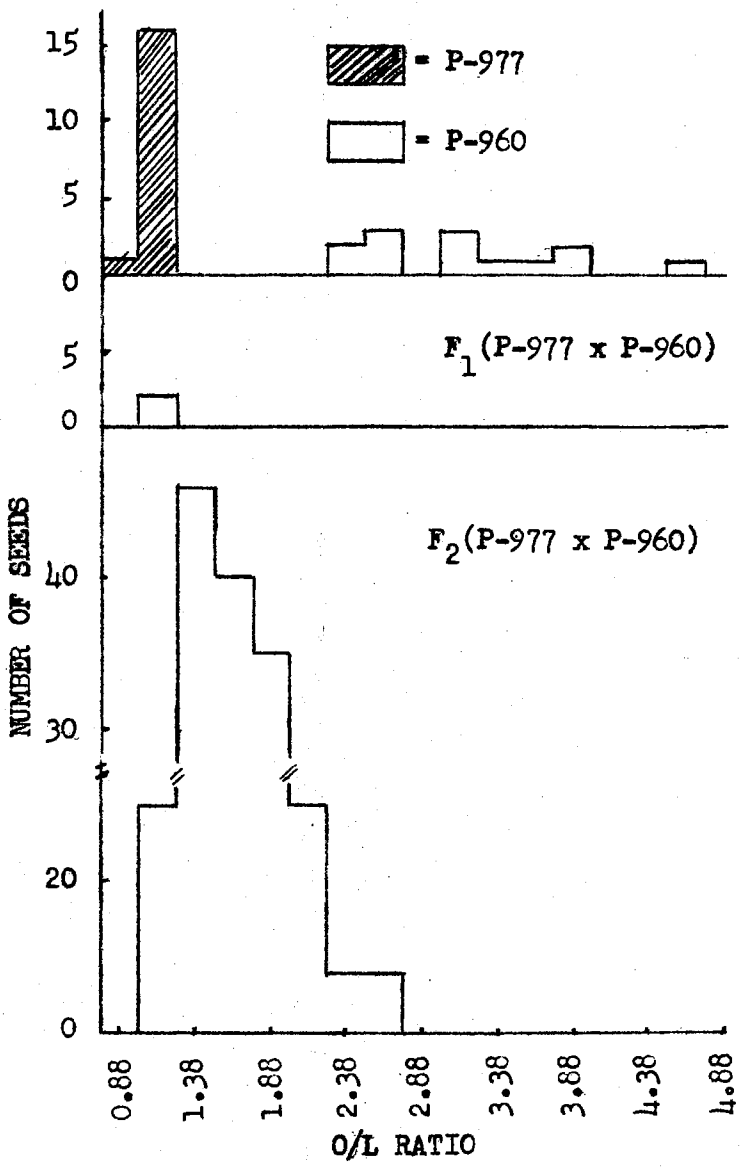

Figure 15. Frequency Histogram of O/L Ratios of F_1, F_2, and Parents of Cross P-977 x P-960 Grown in the Greenhouse

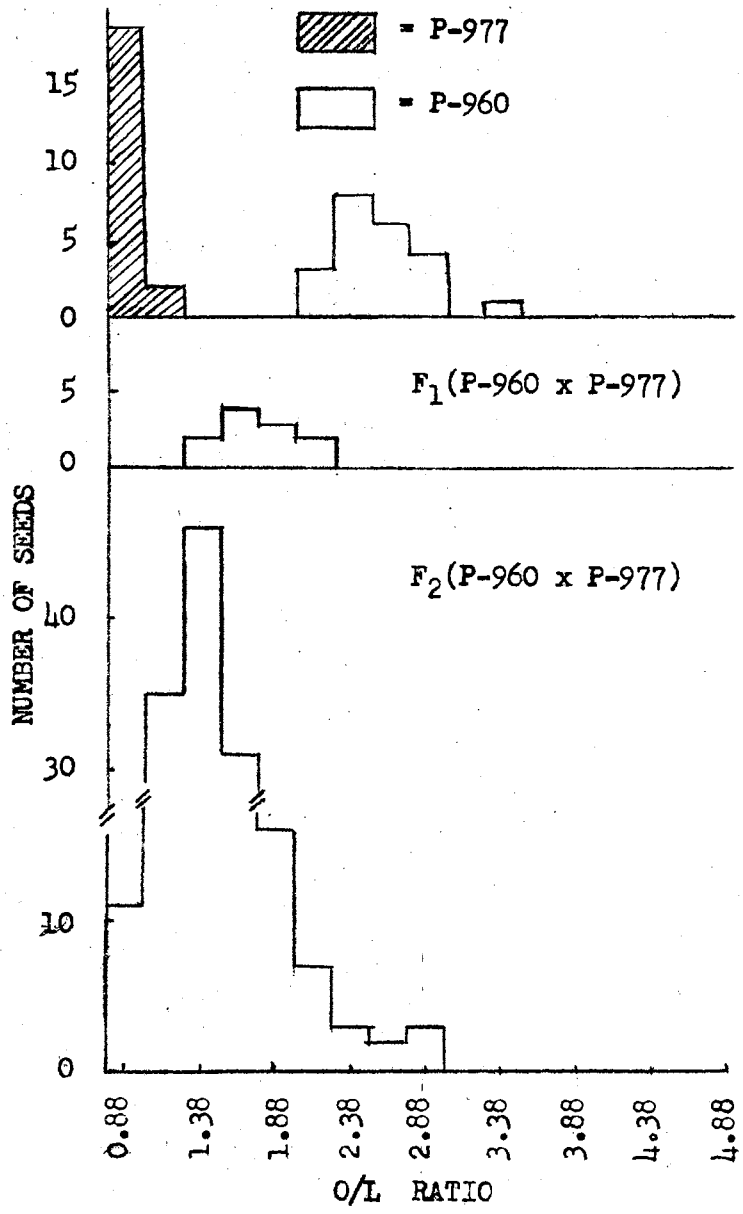

Figure 16. Frequency Histogram of O/L Ratios of F_1, F_2, and Parents of Cross P-960 x P-977 Grown in the Field

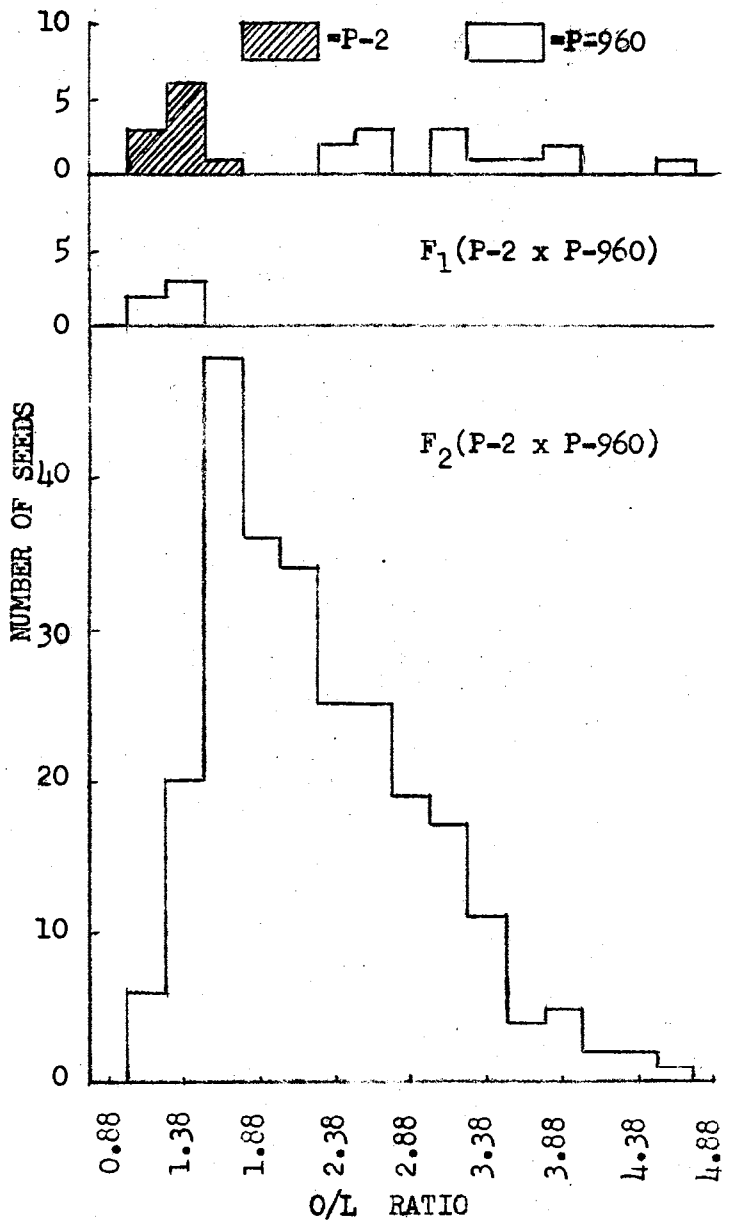

Figure 17. Frequency Histogram of O/L Ratios of F_1, F_2, and Parents of Cross P-2 x P-960 Grown in the Greenhouse

populations of P-977 x P-960 indicate that the greenhouse and field conditions and the direction of the initial cross did not significantly influence the segregation pattern or the frequency distributions of O/L ratios in the F_2 populations.

In "low" O/L cultivar x "high" O/L cultivar crosses, such as P-2 x P-963, P-2 x P-1618, and P-1616 x P-972, the frequency distributions of the F_2 populations were fairly normal (Figs. 10, 11, and 18). The backcross of F_1(P-2 x P-963) x P-2 also showed a fairly normal distribution, but when the F_1(P-2 x P-963) was used as a pollen parent to backcross on P-2, the BC_1 population tended much closer to the P-2 parent. In two backcrosses, P-2 x F_1(P-2 x P-1618) and P-1618 x F_1(P-2 x P-1618), the BC_1 populations displayed fairly normal distributions but both of them tended toward the "low" O/L parent, P-2. The BC_1 population of P-1618 x F_1(P-2 x P-1618) had a little wider range of O/L ratios than that of P-2 x F_1(P-2 x P-1618).

Fig. 19 shows an overlapping of the frequency distributions of P-960 and P-1617. The F_1 mean was higher than its "high" O/L parent, P-1617. The frequency of the F_2 population showed more than one peak and did exhibit transgressive segregation.

In the present study, only two samples of F_2 seeds, from the crosses P-960 x P-2 and P-977 x P-2, were available to develop F_2 plants and F_3 seeds on the Perkins Agronomy Research Farm. Their frequency histograms were shown in Figs. 6 and 8. Both F_2 populations from P-977 x P-2 showed a normal distribution. The F_3 seed population and F_2 plant mean population from P-960 x P-2 segregated much as the F_2 seed population and were skewed toward the "low" O/L parent, P-2. Transgressive segregation to both lower and higher O/L ratios occurred in

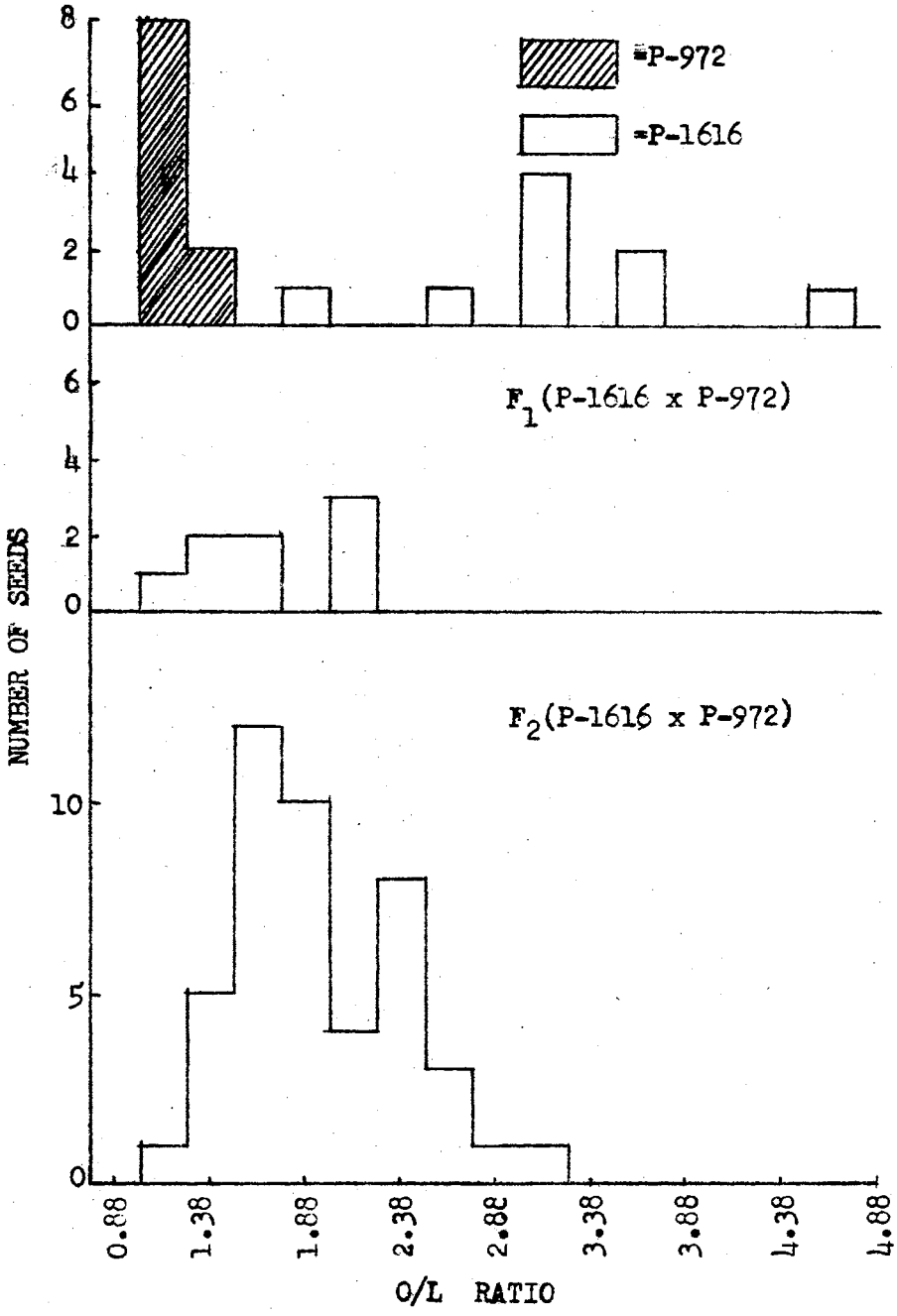

Figure 18. Frequency Histogram of O/L Ratios of F_1, F_2, and Parents of Cross P-1616 x P-972 Grown in the Greenhouse

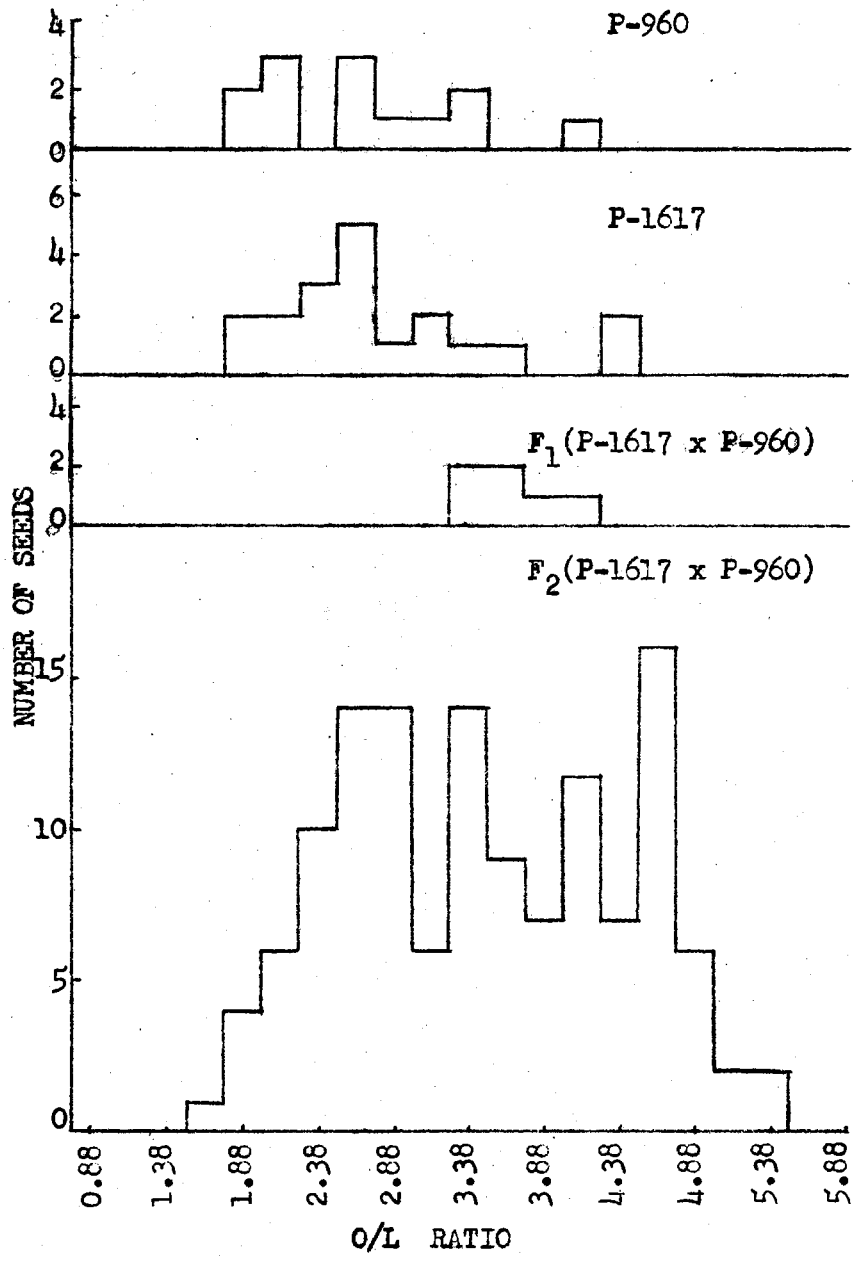

Figure 19. Frequency Histogram of O/L Ratios of F_1, F_2, and Parents of Cross P-1617 x P-960 Grown in the Greenhouse

the F_2 and F_3 seed generations but not in the F_2 plant mean for the cross of P-960 x P-2.

The O/L ratio data from F_1, F_2, F_3 and BC_1 populations show that the O/L ratio is inherited quantitatively and is controlled by multiple genes. It would be helpful to understand this character by computing its heritability and the possible number of genes involved. The heritability (h) of a character can be estimated by the following formulae (7):

$$h = \frac{V_{F_2} - V_{F_1}}{V_{F_2}} \times 100, \text{ or}$$

$$h = \frac{V_{F_2} - (V_{P_1} + V_{P_2})/2}{V_{F_2}} \times 100$$

where; V_{F_2} = variance of F_2 population, V_{F_1} = variance of F_1 population and V_{P_1} and V_{P_2} are the variances of the two parental populations. Both formulae were used in the present study depending upon the availability of the appropriate variances. Variances of parental and F_1 populations were computed from the 1969 crop grown in the greenhouse. All F_2 populations were grown in the same greenhouse in 1970 with the exceptions of P-977 x P-2 and P-960 x P-2, which were grown in the field with their parents. The results are given in Table X.

The possible number of genes (n) controlling inheritance of the O/L ratio were estimated for several crosses by using Wright's formula (7):

$$n = \frac{.25(.75 - h + h^2)D^2}{V_{F_2} - V_{F_1}}$$

TABLE X

ESTIMATE OF THE HERITABILITY AND THE MINIMUM NUMBER OF GENES INVOLVED IN O/L RATIOS FROM VARIOUS CROSSES IN PEANUTS GROWN IN THE GREENHOUSE

Cross	O/L Difference Between the Two Parents	Heritability (%)	Minimum No. of Genes
P-2 X P-977	0.24	88.71	1
P-2 X P-960	1.85	95.57	2
P-2 X P-963	2.41	75.57	3
P-2 X P-1618	2.39	83.45	7
P-977 X P-2*	0.23	81.38	-
P-977 X P-960	2.09	94.20	7
P-977 X P-1618	3.63	29.82	4
P-960 X P-2*	1.39	29.24	-
P-1616 X P-977	2.09	95.88	2
P-1616 X P-972	2.00	22.86	17

* These were grown in the field at the Stillwater Agronomy Research Station

where: $h=(\overline{F}_1 - \overline{P}_1)/(\overline{P}_2 - \overline{P}_1)$, $D=\overline{P}_2 - \overline{P}_1$, $\overline{P}_1$ = the mean of the lower O/L parent, $\overline{P}_2$ = the mean of the higher O/L parent, $\overline{F}_1$ = the mean of the F_1 population, and $\overline{F}_2$ = the mean of the F_2 population. For unbiased estimates of the gene number, there are several assumptions to be met as pointed out by Burton (7). The results of the computation are presented in Table X.

Most of the crosses appeared to have a very high heritability of the O/L ratio in the F_2 generation. The estimates of the minimum number of genes involved in the inheritance of O/L ratio ranged from one gene to seventeen in the various populations. The results from F_1, F_2, F_3, and BC_1 also suggested that the inheritance of O/L ratio in peanuts is probably controlled by a complex genetic constitution. The present results also confirm the conclusion which was made earlier by Mason and Matlock (42).

The O/L ratio represents only the relative amount of oleic acid to linoleic acid; it shows neither the percentage of these two acids in total oil content nor the relationship to the other fatty acids. Holley and Hammons (27) reported that oleic acid and linoleic acid content in peanuts were negatively correlated (-0.988). In an individual kernel study, a negative correlation of -0.9645 has been reported (55). Because of this association of oleic and linoleic acids, these two must be closely related in the unsaturated fatty-acid biosynthetic pathway. The present results, however, indicate that inheritance of the O/L ratio appears to be quantitative, rather than qualitative, and controlled by multiple genes. The genetic system for controlling the amount of each acid in peanuts does not appear as simple as has been reported in maize (49) and safflower (37,39).

CHAPTER VI

RELATIONSHIP BETWEEN O/L RATIO AND PEANUT BUTTER SHELF-LIFE

Pickett and Holley (47) found that oil from Spanish type peanuts was more susceptible to peroxide rancidity than that from either the Runner or Virginia types. Fore, et al. (18) found that the relative linoleic acid content is one of the major factors affecting the stability of the oils tested. Holley and Hammons (27) also confirmed that linoleic acid is the predominant factor in peanut oil keeping-time. Mason and Matlock (42) reported that peanut butter from varieties with wide O/L ratio had longer shelf-life. However, there is an exception to these results. Ga. 186-28 (P-972) has a long shelf-life of peanut butter but has a narrow O/L ratio. Further studies on the relationship between O/L ratio and peanut butter shelf-life have been conducted in the present study.

I. Materials and Methods

Sound mature kernels of the ten cultivars from the 1969 genotype x environment interaction study harvested from the Stillwater Agronomy Research Station were used for this study. The peanuts of each cultivar were divided into two samples. The samples varied from 50 to 70 grams depending on the available seeds of each cultivar. Each sample was then made into peanut butter.

To make peanut butter, the raw shelled peanuts were placed in a modified rotisserie oven and roasted to a "golden brown" cotyledon color. The roasted peanuts were split and degermed with a splitter and the testa and germ were removed by hand. The roasted cotyledons were weighed, 0.5 percent salt added and ground into peanut butter using a Quaker City Mill. Each of five taste panel members evaluated the peanut butter samples. A maximum of five samples including a coded standard were compared with a known standard each day with respect to odor, flavor, taste, roast, texture, dryness, and preference. The standard ratings for the organoleptic characteristics of peanut butter used by the Peanut Quality Laboratory, Oklahoma State University, are listed in Table XI. To ascertain the shelf-life of the peanut butter, the samples were allowed to remain on a shelf at room temperature for 0, 85, and 180 days and then re-evaluated by the panelists.

The experiment was a randomized complete block design with two replications. Since 11 cultivars, 3 storages, and 5 taste panelists were involved, this was an 11x3x5 factorial experiment and the analysis of variance was made on that basis.

Peanut butter samples were taken for O/L ratio determination after room temperature storage for 180 and 300 days. Each peanut butter sample was stirred uniformly and two subsamples, approximately one-half gram each, were placed in test tubes and treated with the reagents and procedure mentioned earlier for the rapid O/L analysis. The O/L ratios were not determined at day zero for the peanut butter samples. However, the O/L ratios for 0-day were based on the half-seed analyses. The analyses of variance for the data were conducted according to the

TABLE XI

THE STANDARD RATINGS FOR THE ORGANOLEPTIC CHARACTERISTICS OF PEANUT BUTTER USED BY THE TASTE PANEL IN THE PEANUT QUALITY LABORATORY, OKLAHOMA STATE UNIVERSITY

Odor		Flavor		Taste		Roast		Texture		Dryness	
Rating	Comment	Rating	Comment	Rating	Comment	Rating	Comment	Rating	Comment	Rating	Comment
1	Weak	1	Excellent	1	Sweet	1	Excellent	1	Smooth	1	Moist
2	None	2	Good	2	Fair	2	Good	2	Mealy	2	Moderate
3	Moderate	3	Low	3	Bitter	3	Under	3	Mushy	3	Oily
4	Strong	4	Off	4	Sour	4	Over	4	Chunky	4	Very Dry

factorial arrangement of treatments for the experimental design used. The mean O/L ratios from the peanut butter shelf-life study were compared among the cultivars within storages by Duncan's new multiple-range test (54). The mean O/L ratios were also compared among storages within each cultivar by the same method.

II. Results and Discussion

The peanut butter samples from ten cultivars and a standard were evaluated organoleptically after 0, 85, and 180 days of room temperature storage. As described in Chapter III through V, the O/L ratios of these ten cultivars ranged from "very low" to "high" based on the half-seed analyses. The Spanish cultivar, Spanhoma, grown at Fort Cobb, Oklahoma, in 1968, was used as the standard for organoleptic tests. The mean percentage of panel members scoring peanut butter superior to, equal to, or inferior to the standard, mean preference rank, and the mean ratings of six organoleptic characteristics are summarized in Table XII. The analysis of variance for the preference rank as shown in Table XIII indicates that the cultivars were not statistically different. Neither storage nor taste panel effects were statistically significant. Summaries of preference rank data are presented in Figures 20 and 21. Among the eleven samples, P-1616 and P-112 appeared to have the least desirable rank (score of 3.7) while the standard (also P-112) showed the most desirable rank (score of 1.6). This difference could be due to year and location effects, since P-112 was grown at the Stillwater Agronomy Research Station in 1969 whereas the P-112 used as standard was grown at the Caddo Peanut Research Station in 1968. The standard tended toward a less desirable

TABLE XII

MEAN PERCENTAGES OF PANEL MEMBERS SCORING PEANUT BUTTER SAMPLES SUPERIOR TO, EQUAL TO, OR INFERIOR TO THE STANDARD, PREFERENCE RANK AND MEAN RATING OF OTHER CHARACTERISTICS FOR SAMPLES OF TEN CULTIVARS GROWN AT STILLWATER, OKLAHOMA, 1969, PLUS ONE STANDARD, STORED ON THE SHELF AT ROOM TEMPERATURE AND EVALUATED AT 0, 85, AND 180 DAYS

P-No.	Storage days	Superior to Standard		Equal to Standard		Inferior to Standard		Mean rank	Mean Rating of:					
		Odor	Flavor	Odor	Flavor	Odor	Flavor		Odor	Flavor	Taste	Roast	Texture	Dryness
0002	0	0	10	20	30	80	60	2.5	1.9	2.5	2.2	2.0	1.2	1.8
	85	0	20	60	30	40	50	3.2	2.4	2.2	1.8	2.0	1.1	1.4
	180	80	80	0	0	20	20	2.7	3.6	3.7	3.3	1.7	2.1	2.5
0977	0	0	0	10	20	90	80	3.0	2.8	2.7	2.3	1.7	1.5	1.7
	85	0	10	60	40	40	50	2.3	2.7	2.2	2.2	1.5	1.4	1.4
	180	50	50	30	50	20	0	3.3	3.5	3.7	3.4	1.9	2.3	3.0
0960	0	0	10	20	10	80	60	2.2	3.1	2.0	1.7	1.8	1.5	1.5
	85	10	20	70	50	20	30	2.6	2.0	2.1	1.9	1.8	1.2	1.6
	180	60	60	20	20	20	20	1.7	3.6	3.4	2.8	1.8	1.8	1.8
0972	0	0	10	30	20	70	70	3.3	3.4	2.3	2.3	2.5	1.3	1.4
	85	40	20	30	30	30	50	2.4	3.1	2.3	2.2	2.5	1.4	1.5
	180	80	60	10	10	10	30	3.2	3.7	3.7	3.2	3.6	2.0	2.0
0958	0	0	10	0	20	100	70	2.6	2.5	2.5	1.8	1.4	1.3	1.6
	85	20	30	40	40	40	30	2.4	2.8	2.0	1.6	1.7	1.2	1.4
	180	80	70	20	20	0	10	1.8	3.8	3.7	3.3	1.7	1.8	2.7
0963	0	0	0	0	0	100	100	3.3	3.6	3.0	2.9	1.8	1.2	1.5
	85	0	0	20	20	80	80	3.6	2.9	3.0	2.3	1.5	1.2	1.7
	180	20	40	30	40	50	20	2.3	3.7	3.5	3.1	1.6	2.0	2.3
1616	0	0	0	0	0	100	100	3.7	3.9	3.5	2.5	2.1	1.6	1.6
	85	30	10	20	20	50	70	3.7	3.1	2.9	2.4	2.1	1.3	1.5
	180	60	90	10	0	30	10	2.4	3.8	3.6	3.6	2.7	1.8	2.1
1617	0	0	0	20	10	80	90	3.6	3.0	3.5	2.8	1.6	1.6	1.6
	85	0	0	50	50	50	50	3.0	2.9	2.7	2.6	1.4	1.2	1.6
	180	20	60	50	20	30	20	3.0	3.6	3.8	3.4	2.2	2.0	2.5
1618	0	10	0	40	40	50	60	2.9	2.5	2.8	2.3	1.6	1.2	1.4
	85	20	30	40	10	40	60	2.9	3.0	2.6	2.1	1.7	1.3	1.7
	180	40	60	20	20	40	20	2.1	3.6	3.5	3.2	2.1	1.8	1.9
0112	0	10	0	30	20	60	80	3.7	2.0	2.8	2.4	1.9	1.1	1.6
	85	20	20	40	40	40	40	3.0	1.8	2.1	2.0	1.8	1.3	1.8
	180	50	60	20	20	30	20	2.2	3.5	3.6	3.1	1.9	2.2	2.5
Std.	0	0	0	100	100	0	0	1.5	2.7	1.5	1.5	1.4	1.3	1.6
	85	0	0	87	87	13	13	2.1	2.5	2.1	2.1	1.7	1.4	1.5
	180	3	7	94	90	3	3	3.5	3.9	3.9	3.5	1.9	2.4	3.4

TABLE XIII

ANALYSES OF VARIANCE FOR RATINGS OF PEANUT BUTTER CHARACTERISTICS FROM TEN CULTIVARS GROWN AT STILLWATER, 1969, PLUS ONE STANDARD, STORED ON THE SHELF AT ROOM TEMPERATURE AND EVALUATED AT 0, 85, AND 180 DAYS BY FIVE PANEL MEMBERS

		Mean Squares						
Source	df	Odor	Flavor	Taste	Roast	Texture	Dryness	Pref. Rank
Reps	1	0.6818	0.1939	4.8485	0.1091	3.1.30	0.0273	0.0030
Cultivars	10	3.4206**	2.4939*	1.8181^{NS}	4.1964*	0.3085^{NS}	0.8891^{NS}	4.2152^{NS}
Error (a)	10	0.6618	0.7472	1.8182	1.0158	0.1897	0.7606	3.6696
Storages	2	31.4030**	48.8818**	43.7848**	3.4030^{NS}	18.5818**	27.3576**	4.0939^{NS}
Cult. X Stor.	20	1.2997*	1.2485*	0.6748^{NS}	0.6164^{NS}	0.2785^{NS}	0.8909^{NS}	3.5806^{NS}
Error (b)	22	0.6000	1.5424	1.1697	1.4697	0.2636	0.4667	1.9000
Judges	4	3.5788**	4.3742**	2.4894**	2.6924**	7.7348**	4.1394**	0.1106^{NS}
Cult. X Judge	40	0.3221^{NS}	0.4242^{NS}	0.3477^{NS}	0.4441^{NS}	0.4115^{NS}	0.3194^{NS}	1.0939^{NS}
Stor. X Judge	8	2.1265*	1.7833**	2.9780**	1.3424**	1.4076**	1.4030**	0.3515^{NS}
Cult. X Stor. X Judge	80	0.5148^{NS}	0.4208^{NS}	0.4514^{NS}	0.3766^{NS}	0.2417^{NS}	0.4155^{NS}	1.3798^{NS}
Error (c)	132	1.0076	0.5788	0.6712	0.4500	0.3576	0.4894	1.0227
Total	329	-	-	-	-	-	-	-

NS not significant; * significant at .05 level; ** significant at .01 level.

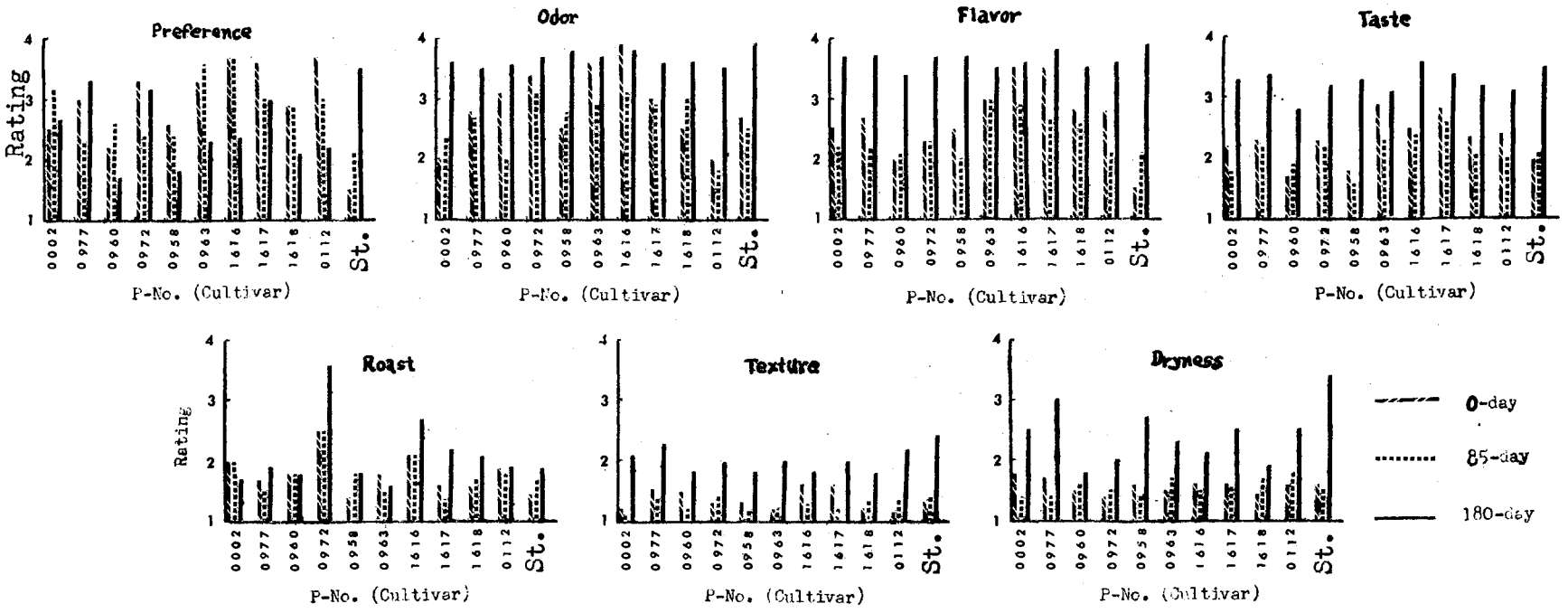

Figure 20. The Mean Ratings of the Organoleptic Characteristics of the Peanut Butter from Ten Cultivars Plus One Standard (St.) Stored for 0, 85, and 180 Days at Room Temperature

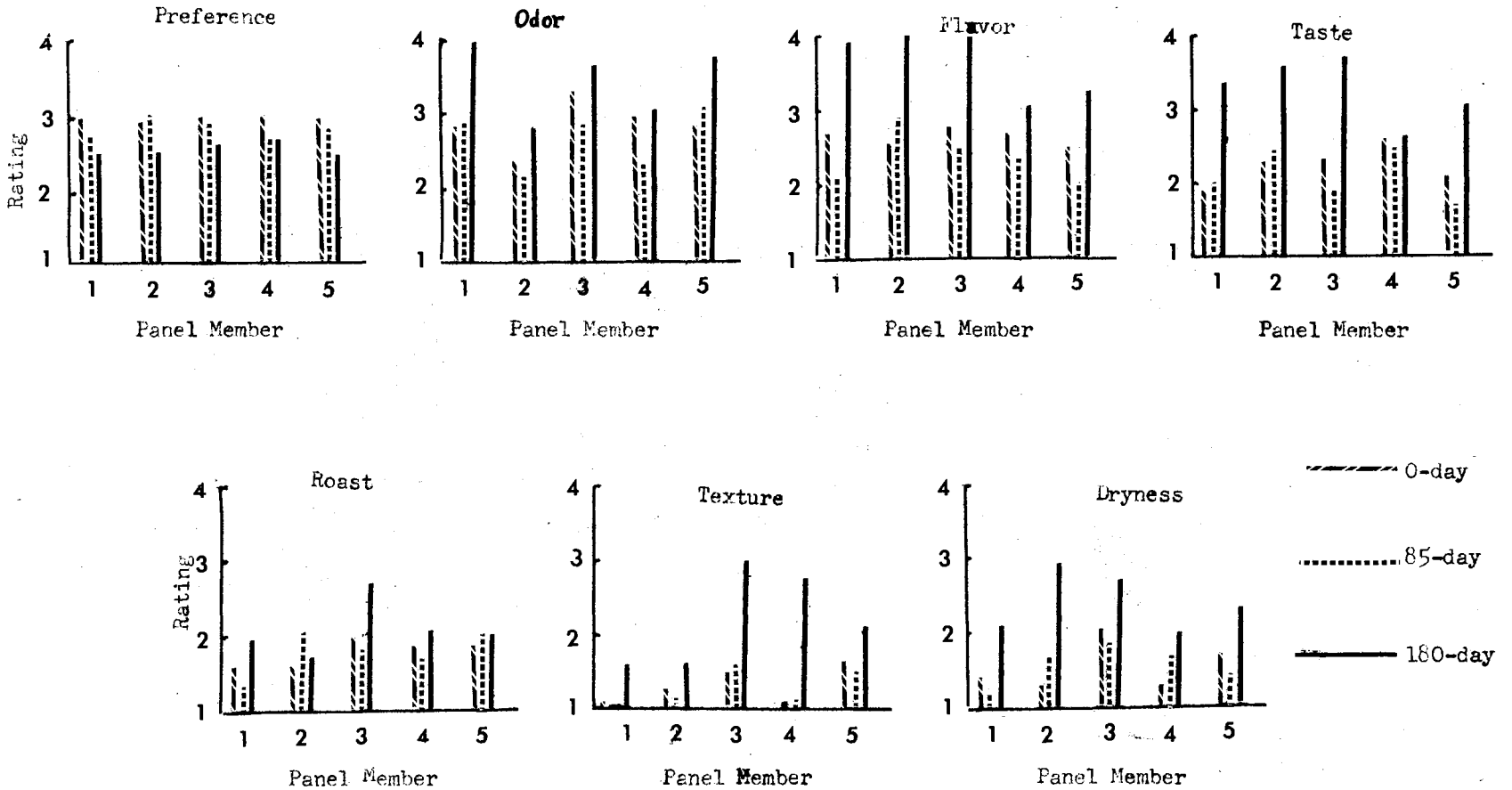

Figure 21. The Mean Ratings of the Organoleptic Characteristics of the Peanut Butter Evaluated by Five Taste Panelists at 0, 85, and 180 Days

rank with longer storage. P-2, which had a "low" O/L ratio, showed a fair rank at both 0 and 180 days (score of 2.5 and 2.7, respectively) but it showed a relatively less desirable rank at 85 days. P-972 and P-977, which had "low" and "very low" O/L ratios, respectively, showed a less desirable rank at 0 day, then improved slightly at 85 days, but went back to the less desirable preference rank at 180 days. Both P-958 and P-960, with a "medium high" O/L ratio, showed a fair rank at 0 and 85 days and improved their rank at 180 days. All four cultivars, P-963, P-1616, P-1617, and P-1618, which had "high" O/L ratios, showed approximately the same pattern with a less desirable rank at 0 and 85 days, but an improvement at 180 days. It should be kept in mind that relatively few observations were obtained for each cultivar, and interpretation of the data should be on that basis.

The average ratings of odor, flavor, taste, roast, texture, and dryness of peanut butter of eleven samples evaluated at 0, 85, and 180 days by the organoleptic panel are also presented in Table XII. Analyses of variance for these organoleptic characteristics are summarized in Table XIII. They indicate that the ratings of peanut butter of the ten cultivars plus the standard were significantly different in odor, flavor, and roast.

All of the organoleptic characteristics of the peanut butter except roast showed highly significant differences between the three storages. Among these characteristics, only odor and flavor appeared to have a cultivar x storage interaction; therefore, these two characteristics of the peanut butter samples did not act independently between variety and storage. However, variety effect was not associated with the storage effect in taste, roast, texture, and dryness. It can

be concluded that the variety effect on odor and flavor of the peanut butter was associated with the storage effect, but the variety effect on the other characteristics was not dependent on the storage effect.

The analyses of variance given in Table XIII indicate that there were differences among the selected cultivars, among the storages, and among the taste panelists for several of the organoleptic characteristics of the peanut butter. To compare their differences, the ratings of the cultivars at 0, 85, and 180-days were plotted for each of the seven organoleptic characteristics in Fig. 20. Most of the cultivars showed a relatively high rating in odor and flavor, a relatively low rating in texture and dryness, and an intermediate in taste and roast, when the peanut butter was evaluated at 0 and 85 days. The mean rating of these cultivars tended to be closer to one another in odor, flavor, and taste after storing for 180 days. Peanut butter from most of the cultivars appeared to have strong odor, off flavor, sour-bitter taste, mealy texture and slightly oily appearance after 180 days according to the taste panelists. Analysis of variance for roast (Table XIII) indicates the storage effects were not significant. Fig. 20 also shows the mean ratings of the three storages for most of the tested cultivars had very similar roast values. This was expected from the normal procedures used in the laboratory to roast peanuts in the preparation of peanut butter. An excellent to good roast is always attempted, thus, a rating of 1 or 2 should always be obtained for "roast" unless something peculiar about a sample prevented a good roast. Fig. 20 shows that the six organoleptic characteristics for most of the cultivars were less desirable at 180 days of shelf-storage than at 0 and 85 days. The 0 and 85 days had a rating very much closer to each other in most

cases. This suggests that these organoleptic characteristics showed a change during the period between 85 and 180 days storage at room temperature. Before the last evaluation of the peanut butter at 180 days, the air conditioner in the Peanut Quality Laboratory was out of order for almost three weeks in the summer of 1970. The change of the peanut butter characteristics could have been significantly accelerated by the increased room temperature (high of 104^{o} F) during that period.

To know the degree of association between the fresh seed O/L ratio and the peanut butter characteristics at 0, 85, and 180 days, the correlation coefficients were calculated and are summarized in Table XIV. The correlation between the O/L ratio and mean preference rank was significant at 85 days, but not at 0 and 180 days. The correlation between the O/L ratio and flavor was significant at both 0 and 85 days, but not at 180 days. The positive correlations indicate that higher O/L ratios resulted in higher flavor ratings which were less desirable. No significant correlations were found between the O/L ratio and the other five organoleptic characteristics. The results did indicate that some of the correlation coefficients were negative, which would indicate a favorable response to the higher O/L ratio, even though they were not statistically significant.

Table XIII indicates that the taste panelists differed significantly in their evaluation of all six organoleptic characteristics of the peanut butter. In no case was the cultivar x judge interaction significant, however, the storage x judge interaction was significant for the six peanut butter characteristics. This significant interaction indicates that the storage effects had a differential effect on the panelists and thus confounded the main effects due to the panelists.

TABLE XIV

CORRELATION COEFFICIENTS BETWEEN THE O/L RATIO OF FRESH SEED AND THE MEAN RATING OF DIFFERENT CHARACTERISTICS OF PEANUT BUTTER OF TEN CULTIVARS GROWN IN STILLWATER, OKLAHOMA, 1969, AND STORED FOR 0, 85, AND 180 DAYS

Peanut Butter Characteristics	Days Stored on Shelf		
	0	85	180
Pref. Rank	0.2818^{NS}	0.6829^{*}	-0.2840^{NS}
Odor	0.5155^{NS}	0.4438^{NS}	0.3599^{NS}
Flavor	0.6375^{*}	0.8600^{**}	-0.3470^{NS}
Taste	0.5007^{NS}	0.5452^{NS}	0.1409^{NS}
Roast	-0.2360^{NS}	-0.3140^{NS}	-0.0590^{NS}
Texture	0.2580^{NS}	-0.2502^{NS}	-0.5826^{NS}
Dryness	-0.3639^{NS}	0.1679^{NS}	-0.4564^{NS}

NS nonsignificant; * significant at the .05 level of probability; ** significant at the .01 level of probability

The ratings for organoleptic characteristics given by the five panelists at 0, 85, and 180 days are shown in Fig. 21. The peanut butter stored 180 days received a higher or less desirable rating from the panelists than 0-day and 85-day storages in essentially every case except roast where the roasting time was purposely adjusted to the individual sample. The higher ratings for odor, flavor, taste, texture, and dryness indicated that the samples were less acceptable to the panelists after 180 days. The ratings given at 0 and 85 days were much closer together and the significant interaction between storage and panelist apparently is the result of nonconsistent ratings for these dates.

The analysis of variance for the O/L ratio of the peanut butter from ten cultivars stored at 0, 180, and 300 days is shown in Table XV. The variances for both cultivars and storages were highly significant. The cultivar x storage interaction was also significant. Since cultivar and storage were confounded by the differential effect of one factor on the other, no comparison among the pooled means was made. Duncan's new multiple-range test was used for making comparisons among the mean O/L ratios of the cultivars at each storage treatment and also among the three storages for each cultivar. The results are presented in Tables XVI and XVII. These data are also plotted in Figure 22. At 0 day, the O/L ratios were based on the half-seed analyses and show that P-112, P-972, and P-977 did not differ significantly from one another. P-112 and P-972 also did not differ significantly from P-2. P-960 had an intermediate O/L value and differed significantly from all other strains. Four "high" O/L ratio cultivars, P-963, P-1616, P-1617, and P-1618, did not differ significantly from

TABLE XV

ANALYSIS OF VARIANCE FOR THE O/L RATIO OF PEANUT BUTTER FROM TEN CULTIVARS STORED AT ROOM TEMPERATURE AND MEASURED AT 0, 180 AND 300 DAYS 1/

Source	df	SS	MS	calculated F
Total	59	88.8957	--	--
Cultivar	9	48.4865	5.3874	16.02**
Storage	2	5.9225	2.9613	8.81**
Cultivar X Storage	18	24.4003	1.3556	4.03*
Error	30	10.0864	0.3362	

1/ The O/L ratios for 0-day were based on the half-seed analyses; the O/L ratios for 180 and 300-days were based on the peanut butter

* Significant at .05 level; ** Significant at .01 level

TABLE XVI

MEAN O/L RATIOS OF PEANUT BUTTER FOR TEN CULTIVARS STORED ON THE SHELF AT ROOM TEMPERATURE AND MEASURED AT 0, 180, AND 300 DAYS 1/

P-No.	0-day 2/	180-day	300-day
0002	1.39 bc 3/	1.59 a	2.35 a
0112	1.26 abc	2.28 ab	2.96 a
0977	0.96 a	3.87 d	2.08 a
0972	1.15 ab	1.76 a	1.76 a
0958	1.61 c	1.52 a	1.90 a
0960	2.18 d	2.38 ab	1.95 a
0963	3.44 e	3.10 cd	5.93 b
1616	3.46 e	3.32 cd	5.58 b
1617	3.20 e	3.33 cd	2.58 a
1618	3.38 e	2.82 bc	2.69 a

1/ 0-day peanut butter O/L ratio from standard P-112 (1969 crop, Ft. Cobb) was 1.19 and P-112 (1970 crop, Ft. Cobb) was 1.14.

2/ O/L ratios for 0-day were based on half-seed analyses; 180 and 300 days were based on peanut butter.

3/ Means followed by the same letter within storages are not significantly different at the .05 level of probability.

TABLE XVII

DUNCAN'S NEW MULTIPLE-RANGE TEST FOR MEAN O/L RATIOS OF PEANUT BUTTER FROM TEN CULTIVARS STORED AT ROOM TEMPERATURE AND MEASURED AT 0, 180, AND 300 DAYS

P-No Storage	0002	0112	0977	0972	0958	0960	0963	1616	1617	1618
0-day[1/]	1.39 a[2/]	1.26 a	0.96 a	1.15 a	1.61 a	2.18 a	3.44 a	3.46 a	3.21 a	3.38 b
180-day	1.59 ab	2.28 a	3.87 c	1.76 b	1.52 a	2.38 a	3.10 a	3.32 a	3.33 a	2.82 a
300-day	2.35 b	2.96 a	2.08 b	1.76 b	1.90 a	1.95 a	5.93 a	5.53 a	2.58 a	2.69 a
S. E.	0.1855	0.6803	0.0768	0.0300	0.2287	0.1800	0.7664	0.6066	0.3635	0.0630
C. V.(%)	14.79	44.42	4.72	2.73	19.32	11.72	26.08	20.93	16.88	3.79

1/ O/L ratios for 0-day were based on half-seed analyses; 180-day and 300-day were based on peanut butter

2/ Means followed by the same letter within cultivars are not significantly different at the .05 level of probability

one another but differed from all other cultivars.

After 180 days of storage, the peanut butter O/L ratios still showed differences among the ten cultivars. P-977, which had the lowest O/L ratio at zero day, had the highest O/L ratio (3.87) at 180 days. However, P-977 was not significantly higher than P-963, P-1616, and P-1617, which were considered to be the "high" O/L cultivars in the study. The O/L values of these strains did not change significantly from 0-day to 180-days of shelf-storage and still remained as a group. At 300 days, two cultivars, P-963 and P-1616, increased their O/L ratio close to 6.0. They did not differ significantly from each other, but they did significantly differ from the rest of the cultivars. The other cultivars were not significantly different from one another after the 300 days. P-963 and P-1616 are two different accessions of supposedly the same cultivar. Their response in this study definitely indicates the two accessions are very much alike, if not identical, genetically.

The mean O/L ratios of the ten cultivars at 0, 180, and 300 days are plotted in Fig. 22. The O/L ratios of P-2, P-958, P-960, P-963, P-1616, and P-1617 at 180 days were closer to their respective O/L ratios at 0-day while the 180-day ratios of P-112, P-972, and P-1618 were closer to their 300-day O/L values. The Duncan's new multiple-range test for the mean O/L ratios measured at the three storages within each cultivar is shown in Table XVII. The O/L ratios of P-112, P-958, P-960, P-963, P-1616, and P-1617 each appear considerably different at the three storage dates in Fig. 22, but storages were not significantly different for any of these as shown in Table XVII. Even though actual O/L ratios were different over the three storages for

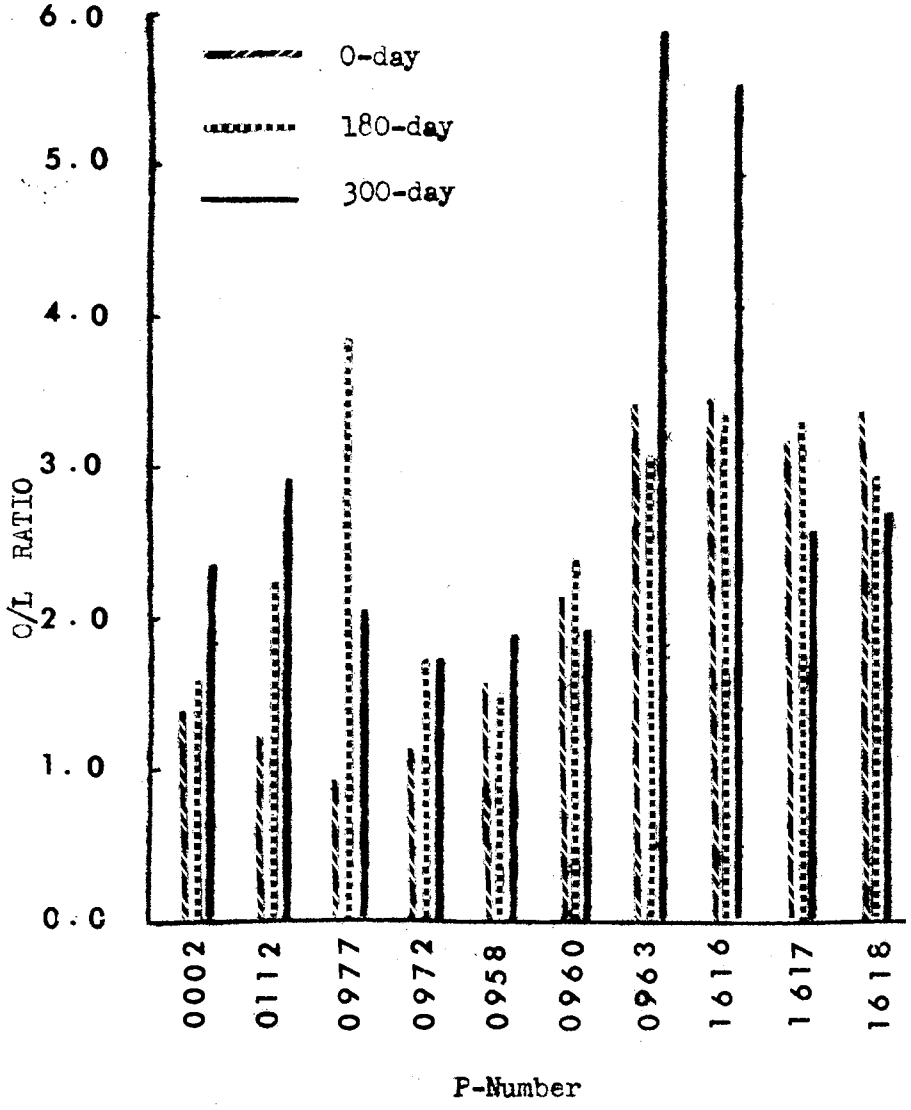

Figure 22. Changes in O/L Ratios of Peanut Butter from Ten Cultivars Stored at Room Temperature and Measured at 0, 180, and 300 Days

P-112, P-958, P-963, and P-1616, fairly high coefficients of variation were obtained resulting in nonsignificant differences statistically. At 0-day, the O/L ratio of P-2 was significantly different from that of 300-day, but not 180-day. The O/L ratio of P-2 at 180-day was not significantly different from that of 300-day. The O/L ratios of P-977 for the three storages were each significantly different from the other. The O/L ratios of P-972 showed that 0-day was significantly different from both 180 and 300 days, but there was no statistical difference between 180 and 300 days.

Correlation coefficients between the O/L ratios of peanut butter determined after the three periods of storage were calculated and are presented in Table XVIII. The correlation between the mean O/L ratios of 0-day and 300-day was significantly high. Correlation values of .46 and .44 were obtained for 0 vs 180 and 180 vs 300, respectively, however, these values did not attain statistical significance.

Fore, et al. (18) reported that Spanish type peanuts have a higher linoleic acid value and the seed oil is less stable than that from either Virginia or Runner type peanuts. Accessions from all of these peanut types were included in the present study. The relative linoleic acid ranged from very high as in P-977 to very low as in the Jumbo type cultivars. This study did show significant cultivar differences in the organoleptic characteristics of the peanut butter, but there was no indication of any relationship between the fresh seed O/L ratio and the stability of the peanut butter stored at room temperature up to 180 days.

Pickett and Holley (47) used peroxide values of oils from raw and roasted peanuts stored at 98^{o} C and found that peroxide developed more

TABLE XVIII

CORRELATION COEFFICIENTS BETWEEN O/L RATIOS OF PEANUT BUTTER SUBJECTED TO THREE PERIODS OF STORAGE

Between	Correlation Coefficient
0 and 180 days	0.4615^{NS}
0 and 300 days	0.6790^{*}
180 and 300 days	0.4410^{NS}

NS nonsignificant; * significant at the .05 level of probability

rapidly in Spanish type peanuts than in Virginia types. Neither the mean ratings for the organoleptic characteristics of the peanut butter nor the O/L ratios from the present studies indicate any trend for the stability to rancidity to be associated with botanical type or the O/L ratio of the accessions used.

Fore, et al. (18) also pointed out that the enhanced stability of the oils from Runner type peanuts may be due in part to a higher tocopherol content. However, no tocopherol contents were determined in the present study.

CHAPTER VII

SUMMARY AND CONCLUSION

Ten peanut cultivars, which had O/L ratios ranging from "very low" to "high", were evaluated at two locations in Oklahoma for a two-year period. The results indicated that the cultivar x year interaction affecting O/L ratios was statistically significant. Plotting the data showed that the significance was caused by different rankings of the "low" O/L ratio cultivars in the two years at each location. However, the actual O/L ratios remained together in the "low" group. The cultivar x location and cultivar x location x year interactions were both small and nonsignificant. Soil type differences in the two test areas appeared to have little effect on the relative performance of the O/L ratio of the cultivars.

It was confirmed that the O/L ratio of the oil in a peanut seed is determined by the genotype of the seed. No apparent and consistent evidence was obtained to support maternal influence on the O/L ratio in peanuts.

Data from F_1, BC_1, F_2 and F_3 populations indicated that the inheritance of O/L ratios in peanuts is controlled by genes acting quantitatively.

Peanut butter from ten cultivars plus a standard were stored on the shelf at room temperature and evaluated organoleptically at 0, 85, and 180 days. O/L ratios were also determined on the peanut butter

samples at 180 and 300 days. This study indicated significant cultivar differences in odor, flavor, and roast of the peanut butter, but there was no indication of any relationship between the fresh seed O/L ratio and the stability of the peanut butter stored at room temperature up to 180 days. Peanut butter could be stored on the shelf at room temperature up to 85 days without significant changes in organoleptic characteristics. Significant changes from desirable flavor to off-flavor did occur between 85 and 180 days. At 300 days, the peanut butter O/L ratios of all cultivars, except P-963 and P-1616, which had O/L ratios close to 6.0, ranged from 1.76 to 2.96 but did not differ significantly.

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