Possible Reproductive Factors Contributing to Outcrossing in Peanut (*Arachis hypogaea* L.)[1]

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ABSTRACT

Several peanut (*Arachis hypogaea* L.) breeding lines in the Florida program were highly variable even after constitution from single plant selections after 21 generations of assumed self-pollination. To assess potential causes of this variability a 3-yr study was conducted to determine outcrossing using Krinkle as a genetic marker. There was a significant year by genotype interaction in this study. Two breeding lines with virginia botanical types averaged almost 1.5% outcrossing, and in 1990 more than 3% of the progeny from these lines were the result of outcrossing. The breeding line Valencia 803 averaged nearly 8% outcrossing, and a second valencia type, F623, averaged over 4%.

The differences in outcrossing among these four lines did not appear to be related to floral morphology, pollen viability, or stigma receptivity, although delayed anther dehiscence may have contributed to the higher outcrossing in Valencia 803.

Key Words: *Arachis hypogaea* L., groundnut, crossing, hybridization, cross-pollination.

In the Florida peanut (*Arachis hypogaea* L.) breeding program high levels of morphological variability were observed within several advanced lines derived from individual plants that assumedly had been self-pollinated for 21 generations. Because segregation was observed among progeny of off-type plants selected from these lines, outcrossing was suspected as a source of this variation. Most studies of outcrossing in peanut have reported rates of 2% or less (Coffelt, 1989; Culp *et al.*, 1968; Gibbons and Tattersfield, 1969; Hammons, 1964a; Hammons and Leuck, 1966; Nigam *et al.*, 1990; and Stone *et al.*, 1973). Several references have been made to high outcrossing rates of more than 6%, but these studies have either included very small sample sizes (Bolhuis, 1951, cited in Gibbons and Tattersfield, 1969) or have been anecdotal references, with no details of the experiment (Leuck and Hammons, 1969); and Hammons 1963).

In one study (Dutta *et al.*, 1987), investigators examined induced mutations as a method for enhancing cross-pollination in peanut, and identified a line with 20.8% outcrossing. The authors indicated, however, that it was difficult to distinguish between variability caused by cross-pollination and variability caused by mutagenesis.

Researchers have found fluctuations in outcrossing rates attributable to both environmental variation and differences among peanut genotypes. While most investigators have suggested that varying insect population levels were responsible for different outcrossing rates among locations and among years, few researchers have studied the possible causes of different rates among genotypes. Bolhuis *et al.* (1965) suggested that variation in time of anther dehiscence may be responsible for differences among genotypes. Leuck and Hammons (1969) thought different proportions of abnormal flowers among genotypes could alter outcrossing frequency.

This study was designed to determine if variation in the unstable breeding line, Valencia 803, was caused by high outcrossing frequencies. We also examined additional breeding lines to assess genetic variation for outcrossing frequency. Differences in floral morphology and reproductive characteristics were examined to determine if these traits contributed to variability in outcrossing frequency among genotypes.

Materials and Methods

Four Florida peanut breeding lines derived from pedigree selection were used in this study. These lines were Valencia 803, a valencia botanical type (*Arachis hypogaea* subsp. *fastigiata* var. *fastigiata*) that maintained high variability after single plant selections in advanced generations; F623, also a valencia type; and two virginia botanical types (*A. hypogaea* subsp. *hypogaea* var. *hypogaea*), F636 and F655. F636 is a large-seeded line with a bunch growth habit and F655 is a runner market type with a procumbent growth habit. Assuming self-pollination each generation, Valencia 803 was in the $F_{21:23}$ generation, F623 in the $F_{9:11}$, F636 in the $F_{8:10}$ and F655 in the $F_{7:9}$ generation at the beginning of this study.

Seed were planted in rows 90 cm apart with 30 cm separating seed within rows. Two-row plots were 6.1 m long. Krinkle, a leaflet mutant controlled by a single dominant gene (Hammons, 1964b), was used as a genetic marker. The line containing the Krinkle mutant, a spanish botanical type (*A. hypogaea* subsp. *fastigiata* var. *vulgaris*), was planted in one row. The second row contained one of the four breeding lines, with Krinkle planted alternately between each breeding line within the row. Plots were arranged so that every other row was planted with Krinkle. This arrangement enabled plants of each line to be surrounded on all four sides with Krinkle. The experimental design was a randomized complete block with 14 replicates. Seed were planted on 23 May 1988, 1989, and 1990. At harvest, non-Krinkle plants were separated manually from each plot based on vegetative differences. Further discrimination between Krinkle and the non-Krinkle genotype could be made from pod size, seed number per pod, and testa color.

Seed from each line and each replicate were planted in early April the year after harvest. Seed dryer malfunctions destroyed seed of F636 and F655 from the 1989 crop year. Four weeks after emergence the seedlings were counted and Krinkle phenotypes, representing outcrossed seeds, were determined.

Floral morphology, including standard width and height and hypanthium length, was measured on 10 random flowers on each of 10 random plants from each replication of field-grown plants each year of study. Pollen viability, stigma receptivity, and anther dehiscence also were measured in the greenhouse. Seed of the five genotypes (Valencia 803, F623, F636, F655, and Krinkle) were sown in 30-cm diameter plastic pots on April 12, 1990. Pollen viability was measured when daily flower production per plant reached a maximum. Ten flowers from each plant of each cultivar were harvested each hour from 0600 to 1200 h (EDT) on 24, 26, and 27 May. Pollen was removed from the anthers and pollen viability measured *in vitro* using the technique of Faucette and Emery (1974).

The duration of stigma receptivity was estimated after emasculating and pollinating flowers over a three-week period, using the artificial hybridization technique of Norden (1980). Flowers from six plants of each genotype were emasculated the evening before pollination. Flowers were pollinated at five times the next day, at 0600, 0730, 0900, 1030, and 1200 h EDT. Up to 12 flowers per plant were pollinated each day, depending on the genotype and amount of cloud cover. To preclude confounding effects from genetic differences in pollen viability, pollen used for pollinations was

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a mixture from 20 random breeding lines grown in the greenhouse to provide a source of pollen for this study. If peg growth occurred, the stigma was assumed to be receptive. Peg growth determinations were made up to 21 days after pollination. Standard statistical procedures were used to compare treatments, with arcsine transformations used for percentage data.

Results and Discussion

Outcrossing rates

There was a significant genotype by year interaction for outcrossing rates in this study. Therefore, data are presented separately for each genotype and each year (Table 1). Valencia 803 averaged nearly 8.0% outcrossing for the three years of this study. This line had the highest outcrossing rate each year of the experiment, and was higher than rates reported in other similar extensive studies. This rate would explain the high amounts of variability remaining in the Valencia 803 lines in the Florida program that were assumed to be self-pollinated for 21 generations.

Table 1. Outcrossing rates of four peanut breeding lines grown at Gainesville, FL in 1988, 1989, and 1990.

Genotype	Total plants (no.)	Krinkle (no.)	Outcrossing* (%)
1988			
F636	23,200	263	1.13a
F655	14,393	142	0.99a
F623	9,506	303	3.19b
V803	11,868	964	8.12c
1989			
F623	5,260	195	3.71a
V803	7,824	615	7.86b
1990			
F636	9,798	309	3.15a
F655	3,306	111	3.36a
F623	2,383	149	6.25b
V803	3,832	288	7.52b
Totals			
F636	32,998	572	1.73a
F655	18,319	253	1.38a
F623	17,149	647	3.77b
V803	23,524	1,867	7.94c

* Outcrossing rates within years or within totals followed by different letters are statistically different ($P \leq 0.05$) according to Duncan's Multiple Range Test.

The second valencia botanical type in this experiment, F623, averaged over 4% outcrossing and had the second highest rate each year of the experiment. Although we noted few floral morphology differences among the four lines in this study, the sparse branching pattern of valencia botanical types may make flowers more visible for visiting insects than the dense branching of virginia botanical types.

The two virginia botanical types in this test, F636 and F655, have different flower positions. F655 has an extremely prostrate growth habit and flowers protrude from the vegetative canopy, while F636 has a bunch growth habit and flowers are borne within the canopy. Yet both breeding lines has similar outcrossing rates in this study. Rates were near 1% in 1988 and greater than 3% in 1990. The yearly fluctuations in outcrossing observed in this study have been reported in other experiments (Culp *et al.*, 1968; Coffelt, 1989; Leuck and Hammons, 1969; and Hammons, 1964a).

Genetic variation in floral morphology and reproductive traits

There was only a 3-day difference in days to first flower among the five genotypes in this study, with F623 and Krinkle flowering first. Little difference in first flowering date for the remaining three breeding lines was noted. We concluded that days to first flower was not a factor in observed outcrossing variation.

Floral morphology also was measured in the five genotypes in this study, and no significant differences were found in size of the floral parts, including hypanthium length. Pollen viability also was similar for the five genotypes (Table 2). While pollen viability fell from 0600 to 1200 h EDT, no differences were noted among the breeding lines in this study.

Stigma receptivity was studied at five different pollination times (Table 3). Although Krinkle had the lowest rate of successful pollination, and F655 had the highest rate, such differences in stigma receptivity would not appear to be responsible for the differences in outcrossing rates.

During artificial hybridizations, it was noted that Valencia 803 appeared to have more intact anthers before 1030 h than other genotypes. Although the number of plants used was small, Valencia 803 anthers dehisced later than other genotypes (Table 4). Because of the higher proportion of indehiscent anthers, fewer Valencia 803 flowers may be self-pollinated when bee activity is high, causing the increased outcrossing rates in this genotype.

Conclusions

We observed outcrossing rates near 8% for Valencia 803

Table 2. Number of germinating pollen grains from five peanut genotypes at each of seven different hours, May 1990.

Hour (EDT)	Genotype V803*	F623	F655	F636	Krinkle	TOTAL
0600	357	359	349	362	366	1793
0700	328	319	324	322	335	1628
0800	275	292	291	273	270	1401
0900	279	270	268	264	260	1341
1000	185	222	227	215	225	1075
1100	169	163	167	173	164	836
1200	75	72	68	87	78	380

* Differences among genotypes within times are not significant at the 5% level.
V803 = Valencia 803

Table 3. Percentages of successful artificial pollinations from each of five peanut genotypes at five pollination times during May 1990.

Hour (EDT)	Genotype V803*	F623	F655	F636	Krinkle
	%				
0600	14	9	14	0	0
0730	41	47	56	43	33
0900	60	69	80	67	14
1030	31	27	53	20	23
1200	19	30	23	6	0

* LSD (0.05) = 24 for comparisons among genotypes within time of pollination.
V803 = Valencia 803

Table 4. Average number of dehisced anthers from six flowers of each of five peanut genotypes at five pollination times during June 1990.

Hour (EDT)	Genotype V803*	F623	F655	F636	Krinkle
0600	0	2	2	1	3
0730	1	3	3	4	4
0900	2	4	5	5	5
1030	5	5	6	6	5
1200	6	7	8	6	6

* LSD (0.05) = 2 for comparisons among genotypes within time of pollination.
V803 = Valencia 803

in each of the three years of this study. This high rate could be responsible for the variation observed in the line, even in advanced generations. We observed yearly variation in outcrossing among the four lines in this study, with all lines outcrossing more than 3% in 1990. This is a higher outcrossing percentage than reported by most researchers. Given these high rates, the distance between plots of different peanut breeding lines may need to be increased. In addition, close attention to removal of off-type plants must occur when outcrossing rates are this high. Although most studies of floral morphology showed no differences among the four breeding lines and the Krinkle genotype used in this study, the higher outcrossing rates of Valencia 803 may be a result of later anther dehiscence. This characteristic may delay self-pollination until bee activity is high, providing opportunities for cross-pollination.

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