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A RECESSIVE LECTIN-FACTOR IN STREPTOPELIA RISORIA[1]

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LECTINS, phytagglutinins or seed agglutinins are now in common use in blood typing (cf. BIRD 1959; BOYD 1963). A classical example is lectin from lima beans which selectively agglutinates cells of type A of man in contrast to those of type B or O. In 1959, BOYD, GREEN, FUJINAGA, DRABIK, and WASZCZENKO-ZACHARCZENKO reported a possible new blood factor in humans detected by an extract of the peanut, *Arachis hypogaea*. Among many seed extracts tested with a variety of animals (cf. MILLER and MCKEEVER 1964) was a sample of the peanut. Differential agglutination of the erythrocytes in a population of ring neck doves, *Streptopelia risoria*, with peanut lectin was marked. This report describes in full the immunogenetic study previously reported in an abstract (MILLER 1963).

MATERIALS AND METHODS

Peanuts of seven distinct strains were obtained. To make extracts, the seeds were first mashed and ground with a mortar and pestle. Saline (0.9 percent) was added to the ground seeds in a ratio of two volumes of saline to one of ground seeds. The mixture was incubated at room temperature for 24 to 48 hours, reground in the mortar and centrifuged. The supernatant test fluid was recentrifuged and stored frozen. A second yield fom the packed sediment after freezing, thawing, and adding an equal volume of saline was nearly as strong as the original test fluid.

The original birds tested consisted of the dove colony maintained by the author and the Serology Laboratory, University of California, Davis. However, all but three of the dove matings yielding family data were made at Iowa State University.

Embryos and squabs were tested (cf. MILLER 1953b), as well as a species hybrid and backcross hybrids of the dwarf turtle dove, *S. tranquebarica*, with *S. risoria*. Five generic hybrids of *Columba livia* × *S. risoria*, and three of *Zenaidura macroura carolinensis* × *S. risoria*, were also available for tests.

The red cells were washed three times in ten volumes (or the equivalent) of isotonic saline (0.75–0.92 percent), centrifuged, and resuspended in saline to 3 percent by volume. Saline agglutination titer tests were performed in quadrupling dilutions of the seed extract by mixing one drop of red cell suspension with two drops of seed extract in Perspex plastic plates. The test works well at room temperatures (23 to 34°C). The tests were read and recorded three times, at 5 min, 30 min, and 2 to 4 hours.

RESULTS AND DISCUSSION

The red cells of many ring neck doves agglutinated rapidly with the peanut extract, with titers from 1/64 to 1/4096, depending on the strain of peanut. A minority of doves tested were negative or had a slow weak reaction, designated

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TABLE 1

Test of peanut reactivity in ring neck doves, Streptopelia risoria

Mating type male × female	Number of matings	Number of progeny in class P+	P−	
P+ × P+	20	154	0	
P− × P+	28	77	93	
P+ × P−	30	83	91	
P− × P−	13	15	52	
Total	91	329	236	565

P−, showing agglutination at titers only to 1/16, reached at about one hour. The strong reactors, P+, reached complete agglutination within 3 to 5 minutes at a ¼ dilution of the extract, and titered 1/64 at 5 minutes. A very few birds were intermediate in titer.

Results from 91 individual cage matings of ring neck doves are presented in Table 1. There were 20 matings of doves in which both parents possessed the strong positive reaction (P+), including five wherein both doves had known P− parents, and they yielded 154 progeny *all* of which were P+. Twenty-eight matings of P− males × P+ females and 30 reciprocal matings yielded a total of 160 P+ and 184 P− offspring. This is consistent with the interpretation that the parents represented a monohybrid testcross. Thirteen matings of P− × P− doves resulted in 52 P− and 15 P+ offspring. Nine of these 13 matings were segregating for this trait. Moreover, a holding pen, all of which were P− doves, raised seven P+ progeny out of 27 tested.

Unlike the usual inheritance of an antigen, dominant to its absence and codominant to other antigenic factors, parents possessing the strong positive reaction (P+) bred true; while matings of "negative" parents (P− × P−) produced some P+ progeny. Therefore, by this technic, the gene for the lectin-factor P+ is recessive. A recessive blood-group factor is known in sheep (Stormont 1951). Several appropriate tests for sex-linkage were clear in demonstrating that the inheritance was autosomal.

The approximate gene frequency in this colony was .2 for the allele controlling P− and .8 for that controlling P+ before matings were selected on the basis of the peanut reactivity.

The intermediate titers could not be attributed to heterozygotes. Most likely the aberrant titers were due to paratyphoid infection (*Salmonella typhimurium* var. *Copenhagen*). Similar results have been noted in pigeons tested with lima-bean extract (unpublished) and with a species-specific reagent (Miller 1953a). The titer of these intermediate doves that survived returned to normal. This presumed *in vivo* phenomenon is probably related to *in vitro* transformations of red cells (cf. Stormont, Suzuki and Miller 1960).

There were 41 P− birds from unknown or from P− × P− parents which were testcrossed to P+ mates in an attempt to find homozygotes. Of these, 28 proved to

TABLE 2

Test of peanut reactivity in progeny from S. risoria × S. tranquebarica *hybrids backcrossed to* S. risoria

Mating type male × female	Number of matings	Number of progeny in class P+	P−	
P+ × P− (F_1)*	1	13	11	
(BC1) P+ × P+	3	27	0	
P− × P+ (BC1)	2	8	3	
P+ × P− (BC1)	4	7	11	
(BC1) P− × P−	4	6	28	
Total	14	61	53	114

* See Table 3.

be heterozygous by producing at least one P+ progeny. Most of the remaining 13 died from paratyphoid before significant numbers of progeny (over six) were obtained. One female produced seven progeny and one male produced 10 progeny in such testcrosses, all of which were P−. Neither survived to start a possible line of P− homozygotes.

Four dwarf turtle doves, 19 Western mourning doves, one Eastern mourning dove, and many pigeons tested with peanut extract have failed to react at all. Five F_1 *C. livia / S. risoria* were all P−. However, only three of these were known to have a P+ dove parent. Three F_1 mourning dove / ring neck hybrids, whose ring neck parent was P+, were P−. One female F_1–S *tranquebarica/S. risoria*, P−, has produced 24 progeny in a backcross to a P+ normal feathered, blond, male, ring neck dove. This mating plus 13 second-backcross matings, including each peanut reactive combination, have produced 114 progeny (Table 2) whose peanut reactivity is consistent with test results presented in Table 1.

Silky plumage in doves (MILLER 1956) is autosomal. Of six character pairs examined, a linkage of the silky gene, *L*, with that for a species antigen of dwarf turtle dove was detected by using the F_1 family just noted (MILLER 1964). This family represented a sex-linked (COLE 1930) testcross in which the possible phase of linkage of the peanut and feather condition may be represented by $\frac{L\ P^+}{+\ P}$. The 24 progeny fill all the possible classes as indicated in Table 3. Thus, close linkage is unlikely, if the allele controlling P− is at the same locus in both species.

Fourteen testcrosses were made with pure ring neck doves to test linkage of the silky and the peanut-reaction traits. The linkage phase was known in only three of these matings. Further, the paratyphoid epidemic prevented large numbers of progeny from any single family. Nevertheless, no evidence for linkage was noted. Two families managed to produce 17 and 22 offspring, respectively. In each family, the four possible classes were reasonably equally filled.

Thirteen squabs from various kinds of matings and from two hours to 15 days after hatching were tested, ten being P+ and three P−. Forty-four embryos from

TABLE 3

Testcross for linkage of silky and peanut-reactive traits in the family of the F_1 female* S. tranquebarica/S. risoria

Sex	Phenotype of progeny				
	silky P+	silky P−	silky+ P−	silky+ P+	
male	3	2	3	1	
female	5	4	2	4	
Total	8	6	5	5	24

* See Table 2.

a mixture of matings and from four to 14 days of incubation were tested. P+ embryos with red cells reacting as strongly as adult cells were detected throughout the indicated range, and 19 typical P− types were noted in the same incubation range. Apparently, the peanut reactivity is a very pronounced characteristic of the red cells from earliest erythropoiesis. The author now routinely samples the squabs at the time of banding when they are 15 to 40 days old.

Absorptions of the peanut extract with *S. tranquebarica* red cells, with P− and with P+ red cells were made. Phytagglutinins are often difficult to absorb (*cf.* Boyd *et al.* 1959; Goon 1964 and unpublished). In contrast, the P− cells, even from *tranquebarica*, removed all the weak reactivity and left the P+ reaction in nearly unaltered strength. P+ red cells in absorption easily removed all reactivity. Thus, peanut lectin is an excellent reagent for individual differences in doves.

Extracts of seeds from the ginkgo, *Ginkgo biloba*, collected in Ames were found to parallel the peanut reactivity in doves. However, the reactions were weaker and the titer differences smaller. Recently in this laboratory, Mr. James Weber (a National Science Foundation Summer Research participant), discovered another parallel reactivity in lectin from *Bandeiraea simplicifolia*. Further, two additional phytagglutinins reactive with nearly all species of cells tested (unpublished) could be absorbed to yield the same or a related reactive pattern with ring neck dove cells as peanut: i.e., the castor bean, *Ricinus communis*, absorbed by red cells of *livia*, and the scarlet runner bean, *Phaseolus coccineus*, absorbed by human O red cells. Again, the reactions are weak. They show, however, that similar lectin specificities may occur in quite unrelated plants.

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The Iowa Veterinary Diagnostic Laboratory identified the causative organism of the disease outbreak. Miss Bonnie Roberts, Community Blood Bank of Central Iowa, kindly provided outdated human blood.

SUMMARY

Peanut lectin differentially agglutinated red cells of "peanut positive" ring neck doves just as effectively as lima bean lectin agglutinates type A of humans. There were 679 progeny tested from particular matings including a species cross, plus eight hybrids from two generic crosses. The gene for peanut reactivity, contrary to the usual rule in inheritance of antigenic factors, is an autosomal recessive. Its frequency in this colony was 80 percent. Red cells of individuals of any age, embryo to adult, could be typed. A Salmonella outbreak prevented establishment of a homozygous peanut-negative line. Much weaker reactive patterns parallel or related to that of the peanut were found in lectin from ginkgo, from *Bandeiraea simplicifolia*, and after absorptions, in lectin from castor bean and scarlet runner bean.

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