HETEROZYGOSITY AT RED CELL ANTIGEN LOCUS *L* AND FERTILITY IN CHICKENS¹

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A literature review on chicken blood groups provides ample evidence in support of an association between red cell antigens of chickens and performance traits of economic importance such as egg production, hatchability, weight of chicks at 9 to 10 weeks, and liveability of progeny, (see reviews by GILMOUR [1960] and BRILES [1964]). This evidence indicates a selective advantage of heterozygosity for blood group genes. The purpose of the present paper is to record further evidence of a similar advantage of heterozygosity at blood group locus *L* in either parent for fertility.

MATERIALS AND METHODS

Chickens used in this study were from a highly inbred line of White Leghorns, called the R line, described by PEASE (1948), and maintained by annual brother-sister matings at the Agricultural Research Council Northern Breeding Station at Reaseheath, Cheshire, England. According to the blood type at the *L* locus it was possible to arrange the following four mating combinations using 16 cockerels and 48 hens allocated to them at random: (1) heterozygous males $\times$ heterozygous females ($L^1L^2 \times L^1L^2$), (2) heterozygous males $\times$ homozygous females ($L^1L^2 \times L^2L^2$), (3) homozygous males $\times$ heterozygous females ($L^2L^2 \times L^1L^2$), (4) homozygous males $\times$ homozygous females ($L^2L^2 \times L^2L^2$).

Throughout the investigation the cockerels were housed in individual cock cages. The cages measured approximately 18 $\times$ 24 $\times$ 24 inches with wire mesh floors and a wooden perch 2 inches from the floor. The whole cock cage unit was placed in a battery house with the female battery. In order to even out any differences in the amount of light received by the cockerels, their positions in the battery were changed in rotation once weekly. An automatic artificial lighting device was used to provide weekly increments of light from autumn onwards. Feeding was *ad libitum* with breeder's pellets and with water from a drip feeding mechanism. A regular inspection of the comb and wattles was made. None of the birds was affected with excessive comb and wattle development, and they were all able to feed well throughout the experiment. Weekly records were kept of their body weights to serve as an indication of their health. In order to avoid extra handling of the birds, weighing was done immediately after a routine collection of semen.

Females were housed in individual cages of two 3-tiered double sided batteries. Their cages were similar in design to those of the cocks, but smaller and modified for egg production. Feeding and artificial lighting were as for the males.

Artificial insemination was employed in order to avoid any influence of preferential mating. The method of semen collection is described by DE SILVA (1961, 1962) and is essentially the same as that described by LAKE (1957). No precautions were taken against temperature shock to spermatozoa at the time of semen collection in view of the evidence obtained by DE SILVA (1963)

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indicating absence of an effect of temperature shock on the fertilizing ability of fowl spermatozoa. Semen was collected twice weekly from each bird with an interval of 3 to 4 days between collections. The order of semen collection was randomized on each occasion. One collection was used for insemination, and the other for laboratory evaluation of semen quality which was determined by estimating the following semen characteristics from five samples of semen from each cock: density of spermatozoa, percentage "dead" spermatozoa, and resazurin reduction time. Techniques used are described by DE SILVA (1961, 1963a,b). Inseminations were carried out late in the afternoon when nearly all the birds due to lay that day had laid. This part of the day was preferred because of the convincing data reported by POLGE (1955) indicating lowered fertility with inseminations within 15 minutes to one hour before egg laying. No hens were inseminated with an egg low in the oviduct as determined by palpation at the time of insemination. Only fresh undiluted semen was used. The inseminating dose was 0.05 ml. Each female was inseminated once weekly for ten consecutive weeks. Separate inseminating units were used for inseminating hens allocated to each male. The order of inseminating hens within cocks was randomised on each occasion.

Eggs were collected from the second day after insemination until the tenth day after the last insemination. They were stored at 50° to 55° F. Each week's collection was incubated in Multiple incubators. Individual candling was done on the third day and again on the seventh day of incubation. All clears on each day were broken open and examined macroscopically for signs of embryonic development. By the examination following candling on the third day, it was possible to identify fertile eggs, the embryos of which had died as early as a few hours after incubation. Fertility was calculated as the percentage of the ratio: (number of eggs which showed signs of embryonic development)/(total number of eggs set).

RESULTS AND ANALYSES OF DATA

Fertility data are presented in Table 1. Eleven of the females were infertile even when they were test-inseminated with semen from incross males. (The

TABLE 1

Fertility data from mating types: (number of eggs fertile)/(number of eggs laid)

Mating type			
1 $L^1L^2 \times L^1L^2$	2 $L^1L^2 \times L^2L^2$	3 $L^2L^2 \times L^1L^2$	4 $L^2L^2 \times L^2L^2$
11/33	0/4*	3/7	0/11*
0/35*	2/3	21/46	0/13*
7/28	7/8	7/13	0/28
0/6*	2/2	7/8	0/1*
1/1	0/5*	1/3	1/3
4/10	1/12	6/11	0/26*
3/4	1/18	14/22	2/27
1/10	11/26	4/29	5/28
2/12	9/23	0/1*	9/36
4/11	1/11	...	7/17
4/19	1/5	...	1/28
1/2	5/16	...	0/31*
11/32	...	...	0/1*
1/1	...	...	...
Mean percent fertility:			
30.4%	31.1%	44.7%	11.4%
(163 eggs)†	(123 eggs)	(139 eggs)	(167 eggs)

* Infertile hens.

† Figures in parentheses indicate the number of eggs on which the percentage is based.

TABLE 2

Summary of mating types analyses

Comparisons	F ratio	Significance
Between all mating types	4.36	(0.05 > P > 0.01)
Between mating types (1), (2) and (3)	1.18	(Not significant)
Between matings types (1) and (4)	6.54	(0.05 > P > 0.01)
Between mating types (2) and (4)	3.33	(Not significant)
Between mating types (3) and (4)	10.39	(0.01 > P > 0.001)

incrosses used in this experiment were from a cross between two inbred lines C and I described by Pease (1948), and hereafter referred to as CI.) Data from these 11 hens were therefore rejected. Using the remaining data, weighted by the number of eggs laid, statistical analyses were carried out with the fertility estimates as percentages transformed to angles (degrees) so that the normality of distribution required for the analysis of variance should be more nearly satisfied. The analyses showed a significant difference between the mating types ($0.05 > P > 0.01$). Further analysis of the data showed that while there was no significant difference in fertility between the first three mating types, the fertility of mating type (4) (homozygous $\times$ homozygous) was significantly lower than the others except mating type (2) (Table 2).

Analyses of data obtained on semen characteristics did not show any significant difference in semen quality between homozygous and heterozygous males (Table 3). Neither was there a significant difference in their fertilizing ability as shown by the analysis of fertility data obtained with 48 CI females (Table 4), which were randomly allocated to the 16 males and inseminated with semen from the same semen samples used for the experimental R-line females throughout the experiment.

Data obtained from a subsequent small scale experiment carried out in the next season are shown in Table 5. In this experiment 44 R-line females and 12 males were used. Each male was assigned one or two females at random from each of the blood types. There were two replicates. In the second replicate, the female lots within a blood type were interchanged. Each replicate lasted four

TABLE 3

Summary of estimations of semen characteristics

Semen characteristic*	Homozygous group			Heterozygous group		
	Group mean (n=8)	Range of means within group (n=5)	SE of mean	Group mean (n=8)	Range of means within group (n=5)	SE of mean
Total density (millions per mm ³)	2.51	1.42- 3.66	± 0.24	2.82	2.21- 3.49	± 0.15
Percent dead spermatozoa	7.40	5.60-11.80	± 0.08	7.50	5.50-10.50	± 0.06
Resazurin reduction time (minutes)	8.93	7.17-14.23	± 1.17	7.37	5.79- 9.33	± 1.20

* For none of the three variables was there a significant difference between the groups ($P > 0.05$).

TABLE 4
Analysis of fertility data with CI females

Source of variation	Degrees of freedom	Mean square	Variance ratio
Between males	15	214.3413	5.111 ($P < 0.001$)
Between homozygous and heterozygous groups	1	304.3657	1.463 (Not significant)
Between males within groups	14	207.9110	
Within males	32	41.9245	

TABLE 5
Fertility data from experiment 2

		(Total number of eggs fertile) / (Total number of eggs laid)					
		$\times L^1L^2$ females Replicate		$\times L^2L^2$ females Replicate		$\times L^1L^1$ females Replicate	
Male blood type		1	2	1	2	1	2
Heterozygous (L^1L^2)	1	16/17	6/6	15/16; 9/11	4/11; 5/5	11/11	4/7; 2/3
	2	0/12*	...	11/15	...	0/1	...
	3	4/5	0/5	0/4*	6/13	...	8/9
	4	7/8	...	...	6/10; 1/6	4/5	...
	5	6/7	...	...	...	14/15	...
	6	15/16	9/16	9/9	6/14	3/3	7/18
Homozygous (L^2L^2)	7	...	15/18	9/16	11/13	9/12	7/8
	8	...	0/12*	0/1	6/13	...	0/2
	9	3/8	4/6	...	0/10*	...	...
	10	1/5	3/3	2/8	3/16	1/2	2/4
	11	1/4	1/6	3/4	...	2/2	6/18
	12	14/15	15/17	8/8	5/7	17/18	5/7

* Infertile hens.

weeks with an interval of three weeks between replicates. All techniques were the same as described for the first experiment, except that each female was inseminated twice a week. Unfortunately, owing to the poor laying capacity of the R-line females, the data obtained were insufficient for a complete analysis of variance. There were 22 missing plots (Table 5). Statistical analysis was therefore abandoned, but means were calculated for the different mating types, weighted by the number of eggs laid (Table 6). These means show the same trend of differences in fertility between the mating types as in the first experiment, thus generally confirming the finding that fertility is lowest in the homozygous $\times$ homozygous matings.

DISCUSSION

It is observed (Table 2) that while there was a significant difference in fertility between mating types (3) and (4) (homozygous males $\times$ heterozygous females, and homozygous males $\times$ homozygous females) there was no such difference

TABLE 6

Mean percentage fertility in the different mating types
(According to L locus blood type of male and female)*

Mating types		
1 Het. ♂ × Het. ♀ $L^1L^2 \times L^1L^2$	2 Het. ♂ × Homo. ♀ $L^1L^2 \times L^2L^2$	3 Homo. ♂ × Het. ♀ $L^2L^2 \times L^1L^2$
81.1% (80 eggs)	65.9% (110 eggs)	71.9% (82 eggs)
Mating types		
4 Homo. ♂ × Homo. ♀ $L^2L^2 \times L^2L^2$	5 Het. ♂ × Homo. ♀ $L^1L^2 \times L^1L^1$	6 Homo. ♂ × Homo. ♀ $L^2L^2 \times L^1L^1$
55.0% (86 eggs)	75.4% (72 eggs)	68.3% (73 eggs)

Mating types 1–4 correspond to those of previous experiment; types 5 and 6 are new.

* Weighted by the number of eggs laid.

Figures in parentheses indicate the total number of eggs on which the percentage is based.

between mating types (1) and (2) (heterozygous males × heterozygous females, and heterozygous males × homozygous females), although in both instances the comparison was between homozygous and heterozygous females. The possibility is thus eliminated of an entirely female blood-type effect being responsible for the low fertility of homozygous × homozygous matings. This discrepancy also cannot be accounted for by the difference in male blood-type [males were homozygous in the first comparison, between mating types (3) and (4), and heterozygous in the second comparison, between mating types (1) and (2)], because it is concluded from the fertility data from the incross females that there is no difference in fertilizing power between homozygous and heterozygous males. Further evidence in support of this conclusion is the absence of a significant difference in fertility between mating types (1) and (3), and between mating types (2) and (4), both of which were comparisons between homozygous and heterozygous males. Therefore, it appears to be an interaction between male and female which is responsible for this low fertility in homozygous × homozygous matings, as indicated by statistical analysis which showed a highly significant interaction term ($0.01 > P > 0.001$). This view is favoured by the fact that there is no significant difference in fertility between mating types (2) and (3), (heterozygous males × homozygous females, and homozygous males × heterozygous females).

From a statistical point of view, however, the above argument may be criticised because variation due to males is not removed. This was not possible with the data available, because this experiment was not planned to obtain this information. Fertility data with the incross females were further analysed to find out whether there was any difference in fertilizing power between the males within

the blood types, since we already know that there is no difference between the types. Two analyses were carried out, one for the homozygous group and the other for the heterozygous group. The analyses indicated that there is a significant difference in fertilizing power between the various heterozygous males ($P < 0.001$), and not between the homozygous males. This is exactly the opposite of what is seen when these males were mated to the homozygous and heterozygous groups of females of the R line, i.e., there is a significant difference in fertility between mating types using homozygous males, while there is no such difference between mating types using heterozygous males. This suggests again either an influence of the female or an interaction between the male and the female. Evidence has been obtained from other experiments in favour of both these possibilities, but confirmation is needed.

This observation of low fertility in homozygous $\times$ homozygous matings seems to be in agreement with the findings of BRILES (1960) who reported for the *B* blood-group locus that hatchability of fertile eggs from homozygous $\times$ homozygous matings is lower than those from matings expected to produce 50 percent heterozygotes. Hatchability data obtained by BRILES (1953, 1954, 1956) and by BRILES and KRUEGER (1955) indicate that "the major effect was probably associated with the blood type of the embryos and only secondarily with that of the parent." This explanation is in accord with the fertility differences between mating types observed in this study. Therefore it appears that even for fertility the condition of heterozygosity is more favoured than homozygosity in the potential embryo, no matter whether the embryo was the result of a mating in which both parents were heterozygous, or only one was heterozygous, or one was homozygous for one allele and the other for another allele, for example $L^2L^2 \times L^1L^1$, as in the second experiment.

The above evidence of association of both early embryonic development and hatchability with blood type of the embryo seems to support the finding of a "definite relation between early embryonic development and hatchability" shown by BRONKHORST (1933), OLSEN and MARSDEN (1954) and MUN and KOSIN (1958). Early cleavage stages of some early deads are undoubtedly classified as infertiles, thus accounting to some extent for this generally observed negative correlation between hatchability and infertility, and it is possible that zygotes of a certain genotype are more prone to die at this very early cleavage stage than are others.

The relationship discussed above, i.e. between mating type (according to blood type) and fertility, is similar to that between serum types and embryonic death in Danish cattle breeds described by ANDRESEN, *et al.* (1959). These workers found that the possibility of foetal survival is diminished when both the dam and the embryo possess the *E* gene. In the present study also, there is lowered fertility when the dam and the embryo have the same blood type L^2L^2 , which is the result of the mating $L^2L^2 \times L^2L^2$. The situation in cattle could have been cited as a complete analogy if it had been possible to obtain fertility results also of the other homozygous mating, $L^1L^1 \times L^1L^1$. (This was not possible in the present study owing to lack of L^1L^1 males). If the fertility results of both these homozygous

matings were to be similar, it would then be possible to offer an explanation for this difference in fertility between homozygous $\times$ homozygous mating and the other mating types, on the same lines as suggested by BRANDT (1959) and by PLUM (1959), who have presented results "which suggest that diversity of cellular antigens might be used as an indicator of general genetic diversity between mates" on which "the phenomenon of hybrid vigour apparently depends." No claims are made "that cellular antigens are responsible for fertility and successful embryonic development. However, dissimilarity of antigens may be used as an indicator not only of dissimilarity of genes affecting blood antigens but of dissimilarity of other genes affecting productive traits as well." Such a postulate needs further experimentation. Therefore it is suggested that experiments using the technique of artificial insemination be carried out with large numbers of birds for each mating type, so that sufficient embryos may be obtained for blood typing in order to study whether the two classes of individuals (i.e. homozygous and heterozygous) segregate according to Mendelian proportions.

In conclusion, it may be stated that the results of this study indicate that the blood genotype at the *L* locus may be used as a genetic marker for appropriate manipulation of the breeding system so as to avoid the low fertility effects of the *L* system in poultry breeding schemes using inbred lines.

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SUMMARY

Four mating combinations according to zygosity at red cell antigen locus *L* in highly inbred chickens were compared for fertility using the technique of artificial insemination. These were (1) heterozygous males $\times$ heterozygous females ($L^1L^2 \times L^1L^2$), (2) heterozygous males $\times$ homozygous females ($L^1L^2 \times L^2L^2$), (3) homozygous males $\times$ heterozygous females ($L^2L^2 \times L^1L^2$), and (4) homozygous males $\times$ homozygous females ($L^2L^2 \times L^2L^2$). Mating type (4) was significantly less fertile than the other mating types except (2). The results suggest an interaction between male and female rather than an entirely female influence for optimum fertility. The genotype at the *L* locus can perhaps be used as a marker to avoid low fertility.

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