ASSOCIATION OF BLOOD-GROUP AND PROTEIN POLYMORPHISMS WITH EMBRYONIC MORTALITY IN THE CHICKEN

J. R. MORTON, D. G. GILMOUR, E. M. McDERMID, AND A. L. OGDEN

*School of Agriculture, University of Cambridge, Thornber Brothers, Halifax, Yorkshire, and
Animal Health Trust Farm Livestock Research Centre, Stock, Essex*

Received August 26, 1964

FISHER (1930b) and FORD (1942) showed that the genes controlling stable polymorphism are highly unlikely to be neutral in survival value. Although other selective forces have been suggested (HALDANE and JAYAKAR 1963), the most likely cause of stable polymorphism remains the overdominant effect of the heterozygote on fitness, as originally proposed by FISHER (1930a).

In the simple case of two alleles per locus, there will be a low overall population fitness if many polymorphic loci show appreciable overdominance. Reduction in the frequency of deleterious homozygotes by the evolution of multiple alleles or pseudoalleles allows polymorphism without marked lowering in population fitness. It should be noted that the number of such alleles that can be maintained in any finite population, and hence the maximum reduction in load due to multiple allelism, is limited (KIMURA and CROW 1964). In such a situation the fitness of some homozygotes may exceed that of some heterozygotes, but the overdominance of each heterozygote over its related homozygotes remains clear (MANDEL 1959). Without multiple allelism adequate population fitness can be maintained only if overdominance is small, in which case the number of polymorphic loci is likely to be reduced by random fixation. This may be avoided by interaction between loci, for example such that only the multiple homozygotes are appreciably deleterious. In this situation the small degree of overdominance at individual loci (KIMURA 1956) will be more difficult to detect than the epistasis.

We have thus outlined two types of polymorphism, involving either multiple alleles or multiple loci with epistasis. These are not necessarily exclusive since polyallelic loci may be epistatic. Possible examples of these types are the B blood group system of chickens, which is polymorphic with many multiple alleles (GILMOUR 1960), and the protein variants of chickens, with several polymorphic diallelic loci (LUSH 1964). Our investigation concerns the effects of both these polymorphic sets on hatchability, an important component of fitness.

MATERIALS AND METHODS

The chickens came from a Light Sussex strain referred to by OGDEN, MORTON, GILMOUR and McDERMID (1962) as Thornber strain 6. This has been maintained as a closed flock since 1952, with a total breeding population in each generation varying around 800 birds made up in about 60 single-male matings. Twice in this period the effective breeding population has been reduced by disease outbreaks to a much smaller size, about four males and 12 females. In addition the

strain has been subjected to some mild selection on testcross performance. It is thus only slightly inbred.

The data concerned the birds in the breeding pens in the 1962 hatching season. The females were trapnested and their eggs individually recorded. Before hatching began eggs were sent either to Stock or Cambridge for the electrophoretic determination of egg-white phenotypes. Both males and females were bled and blood-typed for the B system at Halifax.

Electrophoresis: The thin egg-white was subjected to horizontal starch-gel electrophoresis. At Cambridge the gels were prepared from Connaught "Starch-Hydrolysed", at either 12 percent or the makers' recommended concentration whichever was the less, in POULIK's (1957) discontinuous buffer system. Samples were introduced on Whatman 3 MM papers which were removed after 15 minutes running. Electrophoresis was carried out 4 hours at 4 ma/cm². The gels were sliced, stained for 15 min in 0.2 percent Naphthalene Black 10B and 0.2 percent Nigrosin in a mixture of acetic acid, methanol, ethanol and distilled water (1:1:4:4), and rinsed in the same solvent mixture.

At Stock the gels were made from a specially prepared hydrolysed starch at a concentration of 10 percent. POULIK's (1957) discontinuous buffer system was modified in two respects. The reservoir buffer had lithium hydroxide in place of sodium hydroxide, and the gel buffer had 10 percent of the reservoir buffer added to the usual Tris-citrate. Samples were introduced on Whatman 3 MM papers which were removed after 10 minutes running. Electrophoresis was carried out 4 hr at 7 ma/cm². The gels were sliced, stained 10 min in 1 percent nigrosin in acetic acid, methanol, and distilled water (1:5:5), and washed in the same solvent mixture.

Inspection of the gels allowed identification of the egg phenotypes for loci *II* and *III* of LUSH (1961, 1964) and for the Transferrin-Conalbumin (*Tf*) locus of OGDEN *et al.* (1962). Resolution of the various protein bands was somewhat different in the two methods, but previous work (OGDEN *et al.* 1962) had shown that the results were strictly comparable. In the present work it was sometimes necessary to exchange samples in cases of doubtful classification. Phenotypes that were difficult to recognise with one technique were usually clear on the other.

Blood-typing: Previous work had established that four antigens of the B blood-group system (BRILES, MCGIBBON and IRWIN 1950) were segregating within the strain. Iso-immune antisera specific for these antigens (numbered B₃₅, B₃₆, B₃₇ and B₃₈) were made by appropriate immunisations within the strain, and those used for testing were selected to give strong and clear-cut positive or negative reactions at the appropriate dilutions, without absorption. Agglutination tests were carried out in 10×50 mm test tubes. One drop (approximately 0.025 ml) of diluted anti-serum and one drop of an approximately 4 percent suspension of washed red cells in 0.95 percent saline were mixed together by shaking and allowed to stand 2 hr at room temperature (18 to 22°C). Readings were made according to the macroscopic appearance of the sedimented cells as described by GILMOUR (1959), giving a classification into positive or negative. Occasional doubtful reactions were repeated until a clear-cut classification was possible. Appropriate control cell suspensions were included in all tests.

Estimation of day of death: Data on embryonic deaths were obtained in the course of routine reproduction of the strain at Thornbers' hatchery. All eggs were individually pedigreed to dam. The eggs were routinely candled at 18 days of incubation and those showing no development beyond the 12th day were broken open. Those yolks showing no sign of enlargement or organisation of the blastodisc were classified as infertile. The fertile eggs, together with all those which subsequently failed to hatch, were examined for degree of development by Mr. A. J. BYNG, to whom we are greatly indebted. He estimated the day of embryonic death by the following criteria: Day 1: Enlargement and organisation of blastodisc. Day 2: Start of blood formation. Day 3: Advanced blood formation. Day 4: Size of embryo and blood system. Day 5: Start of eye formation. Day 6: Formation of limb buds. Days 7–9: Size of embryo. Day 10: Start of feather formation. Days 11–12: Development of feathering. Days 13–17: Size of embryo. Day 18: Start of absorption of yolk sac. Day 19: Advanced absorption of yolk sac. Day 20: complete absorption of yolk sac, drying of embryo and breaking of air cell. Day 21: Shell pipped.

Analytical methods. Organisation of data: Although the mortality data were assembled by day of incubation, it is necessary for them to be analysed over periods longer than one day. Analysis

FIGURE 1.—Distribution of day of death of embryos (abscissa) based on 4,088 fertile eggs, showing the four component periods of embryonic mortality. EED: Early Embryonic Death; DG1: Dead Germs 1; DG2: Dead Germs 2; DIS: Dead in Shell.

by dams would otherwise entail use of many fractions with zero numerators. A natural grouping into longer periods can be derived from the data, as in Figure 1 showing the distribution of mortality with days based on the total of 4,088 fertile eggs. As in previous descriptions (LANDAUER 1961), three peaks rise above the low rate of mortality of the 6th to 15th days and the 20th day. Mortalities were thus calculated in four periods, as a percent of the embryos alive at the beginning of each period, as follows: *Early Embryonic Deaths* (EED): deaths on Days 1 to 5. *Dead Germs One* (DG1): deaths on Days 6 to 15. *Dead Germs Two* (DG2): deaths on Days 16 to 19 and one half of each death on Day 20. *Dead in Shell* (DIS): one half of each death on Day 20, deaths on Day 21 and those which failed to hatch. In addition, the *Total Embryonic Mortality* (TEM) ascribable to each dam was calculated as a percent of her fertile eggs.

For methods of analysis based on the normal distribution it is necessary both to convert percentage data to angles and to correct for spurious variance due to percentage values of 0 or 100. Following BARTLETT (1937) values of $0/n$ were replaced by $1/(4n)$, and values of n/n by $1 - 1/(4n)$. Since the percentages ascribable to dams are based on denominators varying from 1 to more than 40 it is also necessary to consider weighting. COCHRAN (1943) concluded that an appropriate weighting method in a multiple classification is extremely difficult both to decide upon and to apply, and moreover that if the numbers are not markedly variable no great loss of efficiency accrues from the use of unweighted angles. Since most error arises from the use of percentages based on small numbers, it was decided in this analysis to eliminate all fractions based on less than ten eggs and to give all the remainder equal weight. The number of dams entering into the analysis was thus reduced from 427 to 175. These 175 females were spread over 52 single-male pens, so that effects due to family or environmental differences are unlikely to be confounded with those associated with blood-group and egg-white genotypes.

Choice of method: With a maximum of 175 items of data organized by dams it is not possible to test all the main and interaction effects of the blood group and egg white loci, for there are 270 possible genotypes. It is therefore necessary to attempt some simplification of the statistical problem by considering the biology of the situation.

Most genetical analyses (e.g. CRITTENDEN and BOHREN 1961) have demonstrated that in general the genotype of the dam has a much larger influence on hatchability than that of the zygote. For the three electrophoretic loci there is a physiological connection in that the dam's genotype is expressed in her eggs—in egg albumen for loci *II* and *III* (LUSH 1961, 1964) and in both albumen and yolk for *Tf* (OGDEN *et al.* 1962; WILLIAMS 1962). It was thus decided to make the analysis by dams' egg-white genotypes including interactions between the three loci.

It is not possible to establish such a connection for the *B* blood group, and we thus decided to use our own data to make a distinction between dam and zygote effects. As will be seen in

RESULTS, a first analysis of total embryonic mortality allowing for either dam or zygote effects, or both, showed that only the zygote blood-group genotype had a significant influence on hatchability.

Since we believed that the egg-white genes acted through the dam genotype, and we had found that the blood-group genes acted through the zygote genotype, it was most logical to omit the interactions between egg whites and blood groups reducing the number of parameters to 37.

Blood group analysis: Parameters are set for the relative mortalities of the ten blood-group genotypes, of dams or zygotes or both. Any one of these is set to zero, and the values of the remainder are estimated as follows. The difference between the frequencies of the parameters in the offspring of any one female and the frequencies averaged over all females in the same egg-white genotype is equated to the difference between the transformed mortality of the eggs of that female and the transformed mortality averaged over all females of that genotype. This yields one equation for each female occurring in an egg-white class of more than one; for example, in TEM there are 171 equations. These are solved for the parameters by the standard least squares method (GAUSS 1826). The significance of the effects of the blood groups as a whole on mortality is tested by variance analysis using the reduction in sums of squares due to fitting the parameters. The standard errors of the parameters are given by the least squares method. Since the parameters and their standard errors represent genotype mortalities relative to the one set to zero, it is necessary to repeat the calculation setting each parameter in turn to zero, in order to obtain standard errors for all the possible differences. In practice it is not necessary to test the significance of every difference.

Egg white analysis: There is evidence for departure of the data from orthogonality (uncorrected $\chi^2 = 21$ for 8 d.f.; $P < 0.01$) and it is thus necessary to use a least squares analysis by fitting regressions. A clear and detailed account of this method for the two-factor case is given by HARVEY (1960), from which the extension to the three-factor case with which we are here concerned is simple. In outline, the method consists in setting up regression formulae for a mean value and for parameters for the effects of each possible genotype, considering the loci singly, in pairs, and together. In the present case of a 3^3 factorial experiment there are then 64 equations. These must be reduced to the number of degrees of freedom available, first by estimating in each one-locus set any two parameters relative to the third. This is possible since we are comparing relative mortalities of three genotypes, and thus in each set the products of the parameters and the genotypic frequencies should sum to zero. In the present situation it is most suitably done by estimating new parameters for the effect of each homozygote relative to the heterozygote. Additionally in the two- and three-locus sets the parameters fitted are reduced to those of double or triple homozygotes relative to heterozygotes. Study of the values of these new parameters allows distinction to be made between additive and various forms of nonadditive effects. Thus the one-locus parameters will sum to zero only if the relative effect of the heterozygote is zero, that is, there is no dominance. Similarly the two- and three-locus parameters will sum to zero only if there is no dominance interaction.

The equations are solved by the least squares method, yielding the variance due to fitting parameters. The difference between this variance and that due to fitting any less complete set of parameters is the variance attributable to the parameters omitted. Thus variances are derived for the effects of each locus and of the interactions between loci.

Computation: The EDSAC II electronic computer of the University of Cambridge Mathematical Laboratory was used for all analyses, the necessary programme being written for us by Mr. ROBERT MARRS, of the A.R.C. Statistics Unit, to whom our grateful thanks are due.

RESULTS

Blood groups: Table 1 shows overall blood-group effects estimated by the reduction in variance within dam egg-white genotypes due to fitting blood-group parameters. This is done separately for blood groups of dams, of progeny (assuming normal Mendelian segregation), and of both. The effects of blood groups on Total

TABLE 1

Variance analysis of B blood groups of dams and embryos with total embryonic mortality (TEM)

	SS	df	MS	VR	P
By dams					
Between blood groups	1,985	9	220.50	< 1	...
Within blood groups	33,803	141	239.74		
By embryos					
Between blood groups	4,647	9	516.34	2.34	< .02
Within blood groups	31,140	141	220.85		
By dams and embryos					
Between blood groups	6,854	18	380.79	1.74	< .05
Within blood groups	28,933	132	219.19		
Total					
Within egg whites	35,787	150	238.58	...	...

Embryonic Mortality (TEM) are evidently sufficiently represented by fitting them for the progeny only.

Similar analyses for the four component periods of incubation, but by progeny blood groups only, are given in Table 2. At first sight it appears that further analyses are justified for TEM only. This is however logically unsatisfactory, for an effect on the whole must include an effect on at least one of the parts. Tests of significance of differences in mortality between genotypes were therefore made for all periods. Table 3 lists the blood-group genotypes which significantly differed from at least one other genotype. In both Dead Germs 1 (DG1) and Dead in Shell (DIS) no more comparisons out of the 45 possible show significance than would be expected by chance alone. This would appear to confirm the conclusions from the variance analyses (Table 2) that differences in these periods contribute little to the overall effects of blood groups on TEM. Nevertheless two of the apparently

TABLE 2

Preliminary variance analysis of blood groups with mortality in the four component periods of incubation

	SS	df	MS	VR	P
Early Embryonic Deaths (Days 1-5)					
Between blood groups	1,564	9	173.74	1.24	> .20
Within blood groups	19,725	141	139.89		
Dead Germs 1 (Days 6-15)					
Between blood groups	468	9	51.98	< 1	...
Within blood groups	6,073	112	54.22		
Dead Germs 2 (Days 16-20)					
Between blood groups	1,138	9	126.42	1.39	< .20
Within blood groups	9,532	105	90.78		
Dead in Shell (Days 20-21)					
Between blood groups	319	9	35.49	< 1	...
Within blood groups	3,969	91	43.61		

significant differences in these periods involve genotypes, 36/36 and 35/36 which are significantly very inferior in TEM. (In this and subsequent sections the shortened notation 35 is used instead of B^{35} for the gene responsible for the production of the antigen B_{35} , etc.). 36/36 is also inferior in Early Embryonic Deaths (EED) and DG2 so that we may legitimately conclude that it is inferior throughout incubation, while 35/36 is inferior primarily in DIS. The other clearly inferior genotype, 37/37, shows its effect mainly in DG2.

Egg whites: It is necessary to consider adjusting the data for blood-group effects before analysing for egg-white effects. From the variance ratios in Tables 1 and 2 it is clear that adjustment in TEM will significantly decrease the error, while adjustment in DG1 and DIS will increase it. For EED and DG2 a decision is more difficult. On the one hand, failure to adjust leaves bias because of the known significant blood-group effects; on the other hand, to adjust would introduce bias because the overall blood-group effects are nonsignificant (Table 2). In these two cases both analyses were made: statistical significance is claimed only if the two analyses agree. Table 4 shows egg-white analyses for TEM (adjusted), for DG1 and DIS (unadjusted) and for EED and DG2 (both). Only in DG1 are there statistically significant effects, the main effect of the *Tf* locus, the interaction of *Tf* $\times$ *III* and the 3-way interaction, although the suggestion of an interaction of *Tf* $\times$ *II* in EED is strong. In this method, all effects are estimated independently

TABLE 3

*Differences in mortality (in angles) between superior and inferior blood-group genotypes
(Superior genotypes are listed horizontally; inferior vertically)*

Early Embryonic Deaths						
	36/38	35/38				
38/38	11.4	13.9*				
36/36	25.8*	28.3*				
Dead Germs 1						
	36/37					
35/38	14.4*					
36/36	21.8*					
Dead Germs 2						
	38/38	36/38	37/38	36/37	35/38	35/37
36/36	7.3	11.5	13.5	14.2	18.5*	21.9
37/37	36.6*	40.7*	42.7*	43.5*	47.8*	51.1*
Dead in Shell						
	36/37					
35/36	16.7*					
Total Embryonic Mortality						
	37/38	38/38	36/38	35/38	35/37	36/37
38/38	...	...	13.6*	19.0*	20.5	23.0*
37/38	...	1.4	15.0	20.4*	21.9	24.5
35/35	4.1	5.6	19.1	24.6*	26.0	28.6
35/36	15.0	16.5	30.0**	35.5**	36.9*	39.5**
36/36	25.2*	26.7*	40.2**	45.7***	47.1**	49.7**
37/37	32.9	34.4	47.9	53.3*	54.8	57.4*

* = Significant at .05.

** = Significant at .01.

*** = Significant at .001.

TABLE 4

Variance analyses of three egg-white loci with embryonic mortality

	SS	df	MS	VR	P	SS	df	MS	VR	P
EED unadjusted*						EED adjusted*				
<i>Tf</i>	626	2	312.76	2.20	<.20	589	2	294.37	2.10	<.20
<i>II</i>	517	2	258.47	1.82	<.20	660	2	329.97	2.36	<.10
<i>III</i>	32	2	16.16	< 1	..	138	2	69.03	< 1	..
<i>Tf</i> × <i>II</i>	1,143	4	285.67	2.01	<.10	1,424	4	356.07	2.55	<.05
<i>Tf</i> × <i>III</i>	162	4	40.56	< 1	..	200	4	50.07	< 1	..
<i>II</i> × <i>III</i>	51	4	12.86	< 1	..	187	4	46.87	< 1	..
<i>Tf</i> × <i>II</i> × <i>III</i>	1,523	6	253.82	1.79	<.20	1,114	6	185.74	1.33	..
Error	21,289	150	141.93	...	..	19,725	141	139.89	...	..
DG1 unadjusted						DG1 adjusted				
<i>Tf</i>	369	2	184.68	3.42	<.05	...	...	...	...	..
<i>II</i>	62	2	31.25	< 1	..	...	...	...	...	..
<i>III</i>	170	2	84.99	1.57	..	...	...	...	...	..
<i>Tf</i> × <i>II</i>	253	4	63.13	1.17	..	...	...	...	...	..
<i>Tf</i> × <i>III</i>	885	4	221.37	4.10	<.01	...	...	...	...	..
<i>II</i> × <i>III</i>	45	4	11.36	< 1	..	...	...	...	...	..
<i>Tf</i> × <i>II</i> × <i>III</i>	665	5	132.90	2.46	<.05	...	...	...	...	..
Error	6,540	121	54.06	...	..	...	...	...	...	..
DG2 unadjusted						DG2 adjusted				
<i>Tf</i>	85	2	42.33	< 1	..	80	2	39.80	< 1	..
<i>II</i>	101	2	50.59	< 1	..	213	2	106.54	1.17	..
<i>III</i>	420	2	209.91	2.24	<.20	211	2	105.29	1.16	..
<i>Tf</i> × <i>II</i>	412	4	102.89	1.10	..	435	4	108.68	1.20	..
<i>Tf</i> × <i>III</i>	356	4	88.90	< 1	..	290	4	72.58	< 1	..
<i>II</i> × <i>III</i>	579	4	144.76	1.55	<.20	732	4	182.89	2.01	<.10
<i>Tf</i> × <i>II</i> × <i>III</i>	216	5	43.17	< 1	..	438	5	87.66	< 1	..
Error	10,670	114	93.60	...	..	9,532	105	90.78	...	..
DIS unadjusted						TEM adjusted				
<i>Tf</i>	190	2	94.96	2.21	<.20	1,235	2	617.57	2.80	<.10
<i>II</i>	48	2	24.24	< 1	..	1,046	2	523.21	2.37	<.10
<i>III</i>	22	2	10.95	< 1	..	652	2	325.92	1.48	..
<i>Tf</i> × <i>II</i>	241	4	60.34	1.41	..	1,535	4	383.77	1.74	<.20
<i>Tf</i> × <i>III</i>	145	4	36.27	< 1	..	777	4	194.17	< 1	..
<i>II</i> × <i>III</i>	178	4	44.62	1.04	..	1,114	4	278.51	1.26	..
<i>Tf</i> × <i>II</i> × <i>III</i>	50	4	12.62	< 1	..	2,188	6	364.63	1.65	<.20
Error	4,288	100	42.88	...	..	31,140	141	220.85	...	..

* Use of adjusted and unadjusted data explained in the text.

of all others so that main effects are tested against Error and not against interactions. In TEM there are indications of four effects, although none reaches conventional levels of significance. Among these are two out of the three effects significant in DG1, together with the main effect of locus *II* and the *Tf* × *II* interaction. Examination of the actual values of the fitted parameters, given in Table 5, shows that these effects are consistent throughout incubation, except in DG2. Moreover there is no evidence of dominance or dominance interaction, as would be indicated by appreciable departure of the sum of the estimates in any set from

TABLE 5

Estimated parameters (in degrees) of homozygous genotypes singly, and in two-way and three-way interaction

Homozygote for			EED		DG1 unadj.	DG2		DIS unadj.	TEM adj.
<i>Tf</i>	<i>II</i>	<i>III</i>	unadj.	adj.		unadj.	adj.		
<i>b</i>	.	.	-4.1	-4.0	-2.6	-0.5	+0.5	-3.4	-5.7
<i>a</i>	.	.	+4.7	+5.1	+0.9	-1.1	-2.3	+5.5	+6.1
.	<i>b</i>	.	-3.4	-2.8	-1.6	+1.6	+2.3	-1.1	-3.5
.	<i>a</i>	.	+5.1	+5.9	+1.2	-0.4	-0.6	+2.0	+7.4
.	.	<i>b</i>	+0.9	+1.1	+0.4	+4.2	+3.1	+1.1	+3.8
.	.	<i>a</i>	+0.2	+0.6	+2.8	-2.1	-2.0	-0.2	-0.6
<i>b</i>	<i>b</i>	.	+1.3	+1.5	-1.2	-1.4	-0.2	+1.1	+2.3
<i>b</i>	<i>a</i>	.	-6.8	-7.6	+0.6	+0.6	-0.0	-2.9	-8.3
<i>a</i>	<i>b</i>	.	-0.5	-0.4	-0.5	+5.8	+4.7	+1.3	-2.1
<i>a</i>	<i>a</i>	.	+6.5	+7.6	-2.2	-5.8	-5.2	+2.8	+7.7
<i>b</i>	.	<i>b</i>	-0.3	-0.5	-1.8	+0.4	+1.7	+0.3	-0.7
<i>b</i>	.	<i>a</i>	-1.6	-2.0	-3.7	-1.6	-0.9	-2.5	-3.4
<i>a</i>	.	<i>b</i>	+0.1	+0.6	-2.4	+2.6	+0.4	+1.4	+1.6
<i>a</i>	.	<i>a</i>	+0.7	+1.7	+6.9	-3.0	-3.7	+2.0	+0.1
.	<i>b</i>	<i>b</i>	+1.2	+2.8	+1.8	+0.9	+3.2	-0.3	+4.9
.	<i>b</i>	<i>a</i>	-1.0	-2.6	-1.3	-3.1	-4.4	-1.9	-7.7
.	<i>a</i>	<i>b</i>	-0.1	-0.5	-0.8	-0.7	-1.3	+0.5	-1.4
.	<i>a</i>	<i>a</i>	-0.5	+0.5	+1.0	+5.3	+6.0	+2.1	+3.8
<i>b</i>	<i>b</i>	<i>b</i>	+1.0	-0.1	+1.0	+0.6	-1.0	+0.8	+2.1
<i>b</i>	<i>b</i>	<i>a</i>	-0.8	-0.7	+0.1	+1.5	+2.5	-0.3	+0.3
<i>b</i>	<i>a</i>	<i>b</i>	-3.8	-2.8	-2.2	+2.0	+3.0	-0.3	-3.0
<i>b</i>	<i>a</i>	<i>a</i>	+0.5	+0.3	-1.1	-3.7	-4.8	-1.0	-1.8
<i>a</i>	<i>b</i>	<i>b</i>	+2.1	+4.1	-2.9	-1.3	+2.1	.	+4.4
<i>a</i>	<i>b</i>	<i>a</i>	-4.5	-4.8	.	.	.	.	-7.8

zero. In particular, the parameters estimated for the interactions are not generally positive, as would be expected if multiple homozygotes were deleterious.

DISCUSSION

The only statistically significant effects of the egg-white loci were the three found in the 6 to 15 day period of incubation DG1. This is a period of low mortality and shows relatively little variance between dams, so that small effects are more easily detected. It is then of interest to consider whether the failure of these effects to show in the analysis of the total embryonic mortality is due to large random variation or to opposing effects in other periods. From Table 6, which shows the direction of the effects approaching significance in each period, it can be seen that EED and TEM are in agreement. Consideration of Table 5 with Table 6 suggests that DG1 shares this agreement. In DIS there is no indication of any effects other than of *Tf*, while in DG2 the effects are quite unrelated to those in any other period. We conclude that the egg white loci affect embryonic mortality during EED and DG1, that is, the first 15 days of incubation; but that random variation between dams in the peak periods of mortality (EED, DG2) has prevented statistical significance being reached in EED or TEM.

TABLE 6

Summary of the effects of the egg-white loci

	EED(1-5 days)	DG1(6-15 days)	DG2(16-20 days)	DIS(20-21 days)	TEM(1-21 days)
<i>Tf</i>					
Probability	<.20	<.05	..	<.20	<.10
Superior	<i>b</i>	<i>b</i>	..	<i>b</i>	<i>b</i>
Inferior	<i>a</i>	<i>a</i>	..	<i>a</i>	<i>a</i>
<i>II</i>					
Probability	<.20	..	..	..	<.10
Superior	<i>b</i>	..	..	..	<i>b</i>
Inferior	<i>a</i>	..	..	..	<i>a</i>
<i>III</i>					
Probability	..	..	~.20	..	..
Superior	..	..	<i>a</i>	..	..
Inferior	..	..	<i>b</i>	..	..
<i>Tf</i> × <i>II</i>					
Probability	<.10	..	..	..	<.20
Superior	<i>b/a</i>	..	..	..	<i>b/a</i>
Inferior	<i>a/a</i>	..	..	..	<i>a/a</i>
<i>Tf</i> × <i>III</i>					
Probability	..	<.01	..	..	..
Superior	..	<i>b/a</i>	..	..	..
Inferior	..	<i>a/a</i>	..	..	..
<i>II</i> × <i>III</i>					
Probability	..	..	<.20	..	..
Superior	..	..	<i>b/a</i>	..	..
Inferior	..	..	<i>a/a</i>	..	..
<i>Tf</i> × <i>II</i> × <i>III</i>					
Probability	~.20	<.05	..	..	<.20
Superior	<i>a/b/a, b/a/b</i>	<i>a/b/b, b/a/b</i>	..	..	<i>a/b/a, b/a/b</i>
Inferior	<i>a/b/b</i>	..	..	..	<i>a/b/b</i>

The B blood-group genotypes of zygotes significantly affect the overall embryonic mortality (TEM). Although the effect is not statistically significant in any of the partial periods of mortality, the main contributions to the overall result come from the high-mortality periods EED and DG2. The individual blood-group genotypes fall naturally into three groups. The first contains three generally inferior genotypes: 36/36 which is inferior throughout, 35/36 whose inferiority gradually increases through incubation and 37/37 which is particularly inferior in the period DG2. The second group includes three genotypes 35/35, 37/38 and 38/38 which have intermediate mortalities throughout incubation. And there is a superior group of four heterozygotes 35/37, 35/38, 36/37 and 36/38. With one exception, the apparent superiority of 36/37 over 35/38 in DG1, all the significant differences found were between not within these three groups.

There has been no previous report on any relation between the egg-white loci and hatchability. Earlier work on the effects of blood groups on hatchability (see review by GILMOUR 1960; also ALLEN 1962; ALLEN and GILMOUR 1962) concerned the specialized situations of inbred lines or incrossbreds, and is thus less relevant to the problem of natural polymorphism than is our work.

It was suggested in the introduction that there are two normal types of polymorphism, that involving demonstrable overdominance at a polyallelic locus, and that involving epistasis between several diallelic loci. We must now consider first whether the results of our investigation agree with such a concept, and secondly the extent to which they can explain the maintenance of these polymorphisms.

We have shown that the *B* blood-group locus is generally overdominant in its effects on hatchability. Of the six possible heterozygotes, four are of superior fitness, and none of the four homozygotes is as viable as the whole population. This fulfils the simplest of MANDEL's (1959) necessary conditions for a stable polymorphism, that the viability of each homozygote shall be less than the mean population viability at equilibrium. By contrast the egg-white loci were found to be generally additive in expression and not demonstrably overdominant. Maintenance of the egg-white polymorphisms cannot however be explained by their interaction effects, for these do not show the expected disadvantage of multiple homozygotes, but rather reinforce the selection shown in the additive effects against the *a* alleles; in particular against *T^f*. We are bound therefore to presume the existence of some balancing selective forces in the later life of the chickens.

Although the estimates of mortalities of blood-group genotypes are made only for a small part of the life cycle, it is interesting to see if they are sufficient to maintain the polymorphism. The simple test mentioned previously, that the viability of each homozygote shall be less than the mean population viability at equilibrium, is a necessary but not sufficient condition for a stable polymorphism. It is in fact a corollary of the necessary and sufficient conditions given as expressions (26) and (30) of MANDEL (1959). Calculation shows that the first of these is not fulfilled by the genotypic mortalities found, so that these alone cannot account for any stable equilibrium of the four gene frequencies. We may also calculate the expected change in gene frequencies with time on the assumption that the estimated mortalities represent the only effects of the genes on fitness. 36 will be fairly rapidly eliminated and 37 much more slowly, leading to an equilibrium with the frequencies of 35 and 38 at 0.427 and 0.573 respectively. At this equilibrium the population would be expected to have a hatchability of 71.1 percent instead of the present 67.7 percent.

We have thus obtained evidence that the *B* blood group and the egg-white loci are associated with powerful selective effects through hatchability. In general terms these are consistent respectively with the two types of polymorphism put forward in the introduction, in that the *B* blood groups showed clear overdominance while the egg-white variants showed epistasis without overdominance. They are not however sufficient in themselves to explain the maintenance of the polymorphisms, and we must presume effects in other periods of the life cycle.

SUMMARY

An investigation has been made into the association between the polyallelic *B* blood-group locus, three diallelic egg-white loci, and hatchability in a relatively noninbred Light Sussex strain.—Blood-group genotypes of zygotes but not of dams were associated with marked differences in mortality over the whole incuba-

tion period, and with smaller differences in some parts of incubation, especially the period from 16 to 19 days. The general effect was of overdominance.—Egg-white genotypes of dams were associated with mortality differences, mainly demonstrable in the 6 to 15-day period of low overall mortality. There were additive and additive $\times$ additive interaction effects.—In neither case could the genotypic mortality differences be used to explain maintenance of the polymorphisms.

LITERATURE CITED

- ALLEN, C. P., 1962 The effect of parental *B* locus genotypes on multiple cross performance in chickens. *Ann. N. Y. Acad. Sci.* **97**: 184–193.
- ALLEN, C. P., and D. G. GILMOUR, 1962 The B blood group system of chickens. III. The effects of two heterozygous genotypes on the survival and egg production of multiple crosses. *Genetics* **47**: 1711–1718.
- BARTLETT, M. S., 1937 Some examples of statistical methods of research in agriculture and applied biology. *J. Roy. Statist. Soc.* **4** (Suppl.): 137–170.
- BRILES, W. E., W. H. MCGIBBON, and M. R. IRWIN, 1950 On multiple alleles affecting cellular antigens in the chicken. *Genetics* **35**: 633–652.
- COCHRAN, W. G., 1943 Analysis of variance for percentages based on unequal numbers. *J. Am. Statist. Assoc.* **38**: 287–301.
- CRITTENDEN, L. B., and B. B. BOHREN, 1961 The genetic and environmental effects of hatching time, egg weight and holding time on hatchability. *Poultry Sci.* **40**: 1736–1750.
- FISHER, R. A., 1930a *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.
- 1930b The distribution of gene ratios for rare mutations. *Proc. Roy. Soc. Edinburgh* **50**: 205–220.
- FORD, E. B., 1942 *Genetics for Medical Students*. Methuen, London.
- GAUSS, C. F., 1826 Supplementum theoriae combinationis observationum erroribus minimis obnoxiae (Societati Regiae Exhibitum, 1826). Gottingae 1828.
- GILMOUR, D. G., 1959 Segregation of genes determining red cell antigens at high levels of inbreeding in chickens. *Genetics* **44**: 14–33 ——— 1960 Blood groups in chickens. *Brit. Poultry Sci.* **1**: 75–100.
- HALDANE, J. B. S., and S. D. JAYAKAR, 1963 Polymorphism due to selection of varying direction. *J. Genet.* **58**: 237–242.
- HARVEY, W. R., 1960 Least squares analysis of data with unequal subclass numbers. *Agr. Research Serv. Publ.*, U.S. Dept. Agr. ARS-20-8.
- KIMURA, M., 1956 Rules for testing stability of a selective polymorphism. *Proc. Natl. Acad. Sci. U.S.* **42**: 336–340.
- KIMURA, M., and J. F. CROW, 1964 The number of alleles that can be maintained in a finite population. *Genetics* **49**: 725–738.
- LANDAUER, W., 1961 The hatchability of chicken eggs as influenced by environment and heredity. *Storrs Agri. Exp. Sta. Monogr.* 1.
- LUSH, I. E., 1961 Genetic polymorphisms in the egg albumen proteins of the domestic fowl. *Nature* **189**: 981–984. ——— 1964 Egg albumen polymorphisms in the fowl: loci *II* and *III*. *Genet. Res.* **5**: 39–49.
- MANDEL, S. P. H., 1959 The stability of a multiple allelic system. *Heredity* **13**: 289–302.
- OGDEN, A. L., J. R. MORTON, D. G. GILMOUR, and E. M. McDERMID, 1962 Inherited variants in the transferrins and conalbumins of the chicken. *Nature* **195**: 1026–1028.
- POULIK, M. D., 1957 Starch gel electrophoresis in a discontinuous system of buffers. *Nature* **180**: 1477–1479.
- WILLIAMS, J., 1962 A comparison of conalbumin and transferrin in the domestic fowl. *Biochem. J.* **83**: 355–364.