Genet. Res., Camb. (1970), **15**, *pp.* 265–284
With 1 *text-figure*
Printed in Great Britain

Association of genetic polymorphisms with embryonic mortality in the chicken

II. The B blood-group system and the pure and crossbred progeny of two populations

By D. G. GILMOUR* and J. R. MORTON

Department of Psychiatry and Neurology, New York University School of Medicine, and School of Agriculture, University of Cambridge, England

(*Received* 10 *February* 1969)

1. SUMMARY

We have studied the association of the ten possible *B* blood-group genotypes with mortality during incubation in the 1966 generation of two relatively noninbred populations of Light Sussex chickens. These were related substrains 6D and 6F derived in the 1964 generation by equal division of strain 6, which we had previously studied in its 1962 generation. The *B* blood-group genotypes of zygotes but not of dams were associated with marked mortality differences in 6F, while in 6D almost no differences were found. Comparisons of the findings in 6D and 6F with the results previously published for the ancestor strain 6, after adjustment for overall between-population differences, showed marked changes between 6 and 6F in the mortalities associated with the five most frequent genotypes. In four of these, the mortalities in 6D were about half way between those in 6 and 6F. Taken together, these results provide evidence that the selective pressures on the *B* locus from differential embryonic mortality vary from generation to generation and even between populations differing apparently only slightly in environment and genetic composition.

In progeny of crosses between females from 6D and 6F with males from 5D and 5F respectively, the latter being two related Rhode Island Red/New Hampshire substrains, embryonic mortality was much lower than in the pure matings within 6D and 6F, and only a few associations with *B* blood-group genotypes of zygotes were found.

The results are discussed in relation to the general lack of association of polyallelic polymorphisms with aspects of fitness reported by other workers; and the importance is stressed of studying associations of genotypes rather than genes or serological factors.

2. INTRODUCTION

In a previous paper on this subject (Morton *et al.* 1965) we suggested as a working hypothesis that stable polymorphisms are divided into two major types, not necessarily mutually exclusive, involving *either* rather few loci with multiple

* Research supported in part by U.S. Public Health Service Grant MH-08618.

alleles; *or* many, usually diallelic, loci with considerable epistatic interaction between them. With multiple alleles there is general overdominance for fitness, but polymorphism can occur without much lowering of population fitness, since the frequency of deleterious homozygotes is low. Marked selective effects might therefore be detected at each of the few loci. With many diallelic loci adequate population fitness would be maintained only if the overdominance for fitness at each locus were extremely small, but then the number of polymorphisms would be likely to be reduced by random fixation. This would be avoided if epistatic interactions occurred such that only certain multiple homozygotes were extremely deleterious. We should then expect that the epistasis would be more readily detectable than the small degree of overdominance per locus.

Our results at that time involved mortality during the embryonic period of the chicken, and were thus incomplete as a study of overall fitness. Nevertheless, they gave some support to our hypothesis. As examples of the two types of polymorphism in chickens, we studied the *B* blood-group locus, which is extremely polyallelic (Gilmour, 1960), as contrasted with three diallelic loci affecting proteins of the egg white (Lush, 1961, 1964; Ogden *et al.* 1962). Blood-group genotypes of zygotes but not of dams were associated with marked differences in mortality over the whole incubation period, the general effect being of overdominance. Egg-white genotypes of dams were associated with mortality differences, but only in parts of the embryonic period. Considered singly, the egg-white loci showed rather small additive genetic effects rather than overdominance; but when considered together they showed epistatic effects in both two- and three-way interactions. In no case could the genotypic mortality differences alone account for maintenance of the polymorphisms, and we had to presume that there were balancing effects in other parts of the life-cycle.

We have now made a further study of embryonic mortality in the descendants of the previous birds four generations later. The results for the *B* blood groups are given in the present paper, while those for the egg-white loci will be published subsequently. A surprising new finding has emerged from the comparison of these blood-group results with our previous ones, that the mortalities associated with certain *B* blood-group genotypes have significantly changed, some previously inferior genotypes now being superior, and *vice versa*.

3. MATERIALS AND METHODS

Chickens. We previously studied embryonic mortalities in the 1962 breeding season of a large closed flock of Light Sussex chickens, called strain 6, the history of which was described by Morton *et al.* (1965). Two generations later in the 1964 season this strain was divided into two substrains 6D and 6F. An approximately even division was ensured by allocating an equal number of half-sib groups from each sire family to each substrain to give breeding populations each of a size equal to that of the original strain. They were both kept on the same large farm of Thornber Brothers Ltd., Mytholmroyd, Halifax, Yorkshire, but in separate areas,

so that some differentiation due to 'location' was possible. Our present data came from the birds in the breeding pens in 1965–6, two generations after the division. Each pen contained a single male and up to fifteen females. In addition to the purebred embryos, we also studied crossbred embryos from the same dams mated to sires of two Rhode Island Red/New Hampshire substrains, 5D and 5F. These had been derived in 1964, in a similar way to 6D and 6F, by dividing the original strain 5. This had originated from crosses of Rhode Island Red and New Hampshire and, like strain 6, had been maintained as a large closed flock since 1952 except for two considerable reductions in size ('bottlenecks') due to disease outbreaks. All four substrains were thus no more than mildly inbred. The crossbred data came from crosses of 5D sires and 6D dams, and of 5F to 6F.

Laboratory methods. All laboratory work was carried out in the research laboratory of Thornber Brothers Ltd. under the direction of Mr E. M. McDermid, to whom we are greatly indebted. Sires and dams were blood-typed for the four antigens of the B blood-group system segregating in 6D and 6F (B_{35}, B_{36}, B_{37} and B_{38}) by routine agglutination techniques as previously described. In substrains 5D and 5F there were another four antigens segregating, B_7, B_8, B_9 and B_{10}, and these were similarly identified in sires of crosses with specific iso-immune antisera. No antiserum was available which could detect B_{10} in the presence of B_7, so that where pedigree information could not rule out any confusion between B^7/B^7 and B^7/B^{10} cocks, their progeny were excluded from the data.

Each embryonic death was assigned to a particular day of the incubation period essentially as described before. There were two small differences: (1) Deaths on the first and second days of incubation were grouped together; (2) candling was done on day 13, which improved the accuracy of diagnosis.

Organization of data. The mortality value for each dam was expressed as a fraction, being the number of her embryos dying divided by the number of those at risk. This was calculated, as before, both for the whole of incubation (days 1–21) and for a number of partial periods. Thus the *Total Embryonic Mortality* (TEM) was the fraction of her fertile eggs which died during the whole of incubation (days 1–21). We attempted to derive the partial periods as natural groupings in the data, as follows. Fig. 1 shows the distribution of days of death of 4088 embryos of strain 6 in 1962 (our previous data); of 10762 purebred embryos of substrains 6D and 6F in 1966; and of 26487 crossbred embryos (5D × 6D and 5F × 6F) in 1965–6. For this figure we pooled pures together and crosses together because within pure and within cross matings the two substrains resembled each other closely. The pattern of late mortality in 1965–6 resembled that in 1962, with peaks on days 18–19 and 21, but there was less mortality on days 16 and 17. For the sake of comparability we ignored this minor variation and regarded days 16–19 as a natural period. The late partial periods were accordingly defined as previously, *Dead Germs Two* (DG2): deaths on days 16 to 19 and one half of each death on day 20; and *Dead in Shell* (DIS): one half of each death on days 20, deaths on day 21 and embryos which failed to hatch. The earlier mortality was much changed from 1962 in both pure and cross matings, the peak being reduced and extending over days 4–7

rather than 2–5. The earlier peak in the figure is probably an artifact of the pooling of days 1 and 2. Mortality in the middle period (8–15) was reduced below even the low level seen in 1962, particularly in the crosses. It was thus not possible to make separate analyses of each of the small peaks and periods of low mortality between them, because too many dams had zero mortality values in these short periods. Consequently, the first 15 days were treated as a single period, deaths on these days being called *Early Dead Germs* (EDG). In addition, the main period of increased mortality, days 1–7, was analysed as a separate natural period of some comparability with the Early Embryonic Deaths (EED, days 1–5) of our previous results. We now call these deaths on days 1–7: *First Week Deaths* (FWD). In each partial period the mortality value was expressed as the number of embryos dying divided by the number of those at risk at the beginning of the period. For the analysis, values of $0/n$ were replaced by $1/(4n)$ and values of n/n by $1-1/(4n)$ to correct for spurious variance, and all fractions were then converted to angles so as to render the data approximately normally distributed.

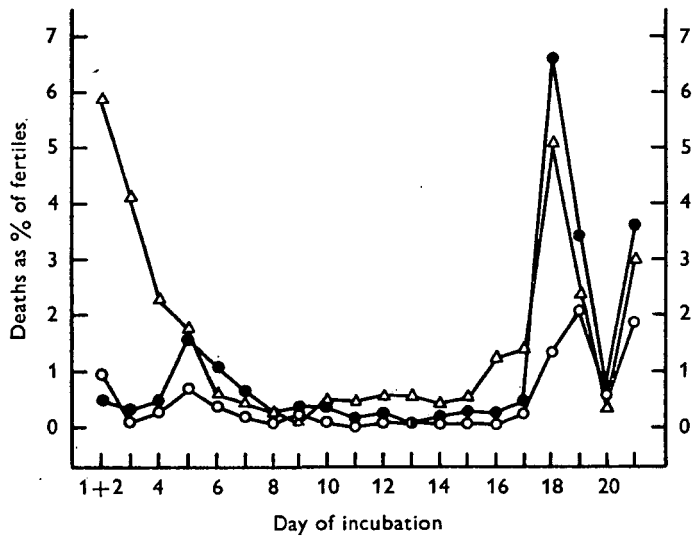

Fig. 1. Distribution of day of death of embryos. △—△, Strain 6; ●—●, Strains 6D+6F; ○—○, crosses.

In considering the question of weighting the angular mortality values we followed Cochran (1943), who 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 results from the use of unweighted angles. We therefore gave equal weight to each mortality value. Since most error due to this procedure arises from fractions based on small numbers, we arbitrarily eliminated from the analyses all dams with less than ten eggs at risk at the start of a period.

The restriction on egg number meant that it was not possible to analyse the weekly hatches separately, and they were pooled over the whole breeding season

(5 weeks for pures, 7 for crosses). Although this cannot invalidate positive findings of blood-group effects, it does raise the possibility that any effects might be less direct, the blood groups of the hens determining the hatches in which they laid their eggs and the hatches having unequal mortalities. This was effectively ruled out by our elimination of hens with less than ten fertile eggs, since these comprised most of the hens which might have contributed to only one or two hatches. From inspection of the distribution of the total number of eggs set from individual hens, it was clear that the majority of hens entering the analyses were in lay throughout the 5 or 7 weeks pooled (Pures: range 10–31, mean and standard deviation 19·4 and 4·3; crosses, 11–42, 31·8 and 5·8).

Choice of analytical method. We wished to test for overall heterozygote advantage at the *B* blood-group locus, but in addition we were influenced by arguments of Mandel (1959) and one of us (Gilmour, 1960, 1962) to expect that differences in selective values of the various heterozygous genotypes might be more marked than any overall heterozygote advantage. We thus required a method of estimating the mean embryonic mortality associated with each genotypic class. There were ten such classes possible from four alleles in adults and embryos of the pure strains, and sixteen (4×4) in the crossbred embryos. With these genotypic mortality estimates, we could then test for both the effects mentioned above, as well as for possible average (or additive genetic) effects of each gene.

We were unable to estimate the interactions between the *B* blood-group locus and the egg-white loci, because this would have involved estimating mortalities for 270 possible genotypic classes from as few as 254 items of data organized by dams. There are reasons for supposing such interactions to be unimportant. Possible genetic influences on embryonic mortality include the effects of the zygote genotype directly, and those of the dam genotype exerted via the egg. The sire genotype is unlikely to have an effect in its own right after fertilization, on the reasonable assumption that the spermatozoa have no physiological function towards the embryo apart from fertilization, and is thus fully accounted for by the genes contributed to the zygote. The egg-white loci presumably have their major expression in the dam on the composition of the eggs laid by her, rather than in the embryo, so that analyses by dams' egg-white genotypes were the obvious ones to make. For the blood groups, the respective genotypes are expressed in both dam and zygote, and thus could possibly affect embryonic mortality either *via* the composition of the egg, or directly in the embryo. We were able to use our own data to distinguish these possibilities, since a first analysis of total embryonic mortality allowing for either dam or zygote effects, or both, showed that only the zygote blood-group genotypes had a significant influence (see Results). We thus made separate analyses by dams' egg-white genotypes and by zygotes' blood-group genotypes, in each case with correction for the other. The egg-white analysis (to be described in a subsequent paper) was made after adjustment of mortalities for the blood-group effects found. In the present blood-group analysis, allowance was made for egg-white effects by making mortality comparisons within dams' egg-white genotypic classes.

Blood-group analysis. For the analysis by dam genotypes, a parameter was set for the mean embryonic mortality of the progeny of each genotypic class of dams. The expected mortality of the progeny of each individual dam was expressed by the parameter appropriate to her genotype. This mortality, as a difference from the average expected mortality of progeny of all dams in the same egg-white class, was equated to the observed differential mortality. There was one such equation for each female occurring in an egg-white class of more than one. Least squares solution of the set of equations gave the reductions in sums of squares due to fitting parameters, thus allowing variance analysis of the overall effects of dams' blood groups on embryonic mortality.

For the analysis by zygote genotypes, a parameter was set for the mean mortality of each genotypic class of zygote. The expected mortality of the progeny of each dam was now expressed as the sum of the products of the appropriate parameters and the zygotic genotypic frequencies expected on normal Mendelian segregation. Any errors in this procedure due to meiotic drive or abnormal segregation could not be excluded. The rest of the calculation was as above. A third similar calculation was made in which parameters for both dam and zygote genotypes were fitted simultaneously.

Since the three variance analyses indicated that the dam effects were unimportant (see Results), further analyses were restricted to zygotes. The least squares solution provided values and standard errors of all the other parameters relative to the one necessarily set to zero. In order to obtain standard errors of all possible paired comparisons between the mean embryonic mortalities associated with zygote genotypes, the least squares calculation was repeated setting each parameter in turn to zero.

Significance tests of genotypic mortality differences. A common problem in making a number of separate significance tests is that some of them are expected to show significance by chance alone, and not because they are truly significant. In these circumstances it is preferable, where possible, to use a multiple significance test, in which the appropriate protection level applies to the collection of tests as a whole and not just to each individual test of a comparison. We used the New Multiple Range Test introduced by Duncan (1955), as modified by Kramer (1956, 1957) to be applicable to means having unequal error variances. This is a test of significance of the ranges of successive extreme values from an ordered arrangement of means. The ratio

$$\sqrt{2} \times (\text{extreme difference}) \div (\text{standard error of difference}) = \sqrt{2}.\text{D/SE}$$

is required to exceed a 'Studentized' significant range, which varies according to the number of means involved and the degrees of freedom of the error term of the analysis of variance. When the value for a paired comparison does not exceed the significant range, that comparison is non-significant, as are all smaller comparisons within the same set of means. Duncan (1957) described a specific way of assembling the values into subsets whose homogeneity was tested, and stressed the need for testing successive extreme ranges in order of size of D/SE rather than D, to ensure

that no significant comparisons within a subset were missed. Because of the form of the analysis, our results were already assembled in subsets appropriate for these purposes, each consisting of a group of estimates of the differences of the other genotypic mortalities from the one set to zero, together with standard errors of these differences. The information on differences was the same in each group; but the standard errors were unique (apart from symmetry) since they depended on which genotype was set to zero. We began by testing the group in which the largest D/SE of the whole series occurred, and continued within the same group until no significance was found. Further groups were tested one at a time, in order of their largest D/SE. Because the original tables (Duncan, 1955) were restricted to 0·05 and 0·01 levels of significance, and in any case contained inaccuracies, we used instead the corrected and extended series of tables provided by Harter (1960).

Comparisons between generations and substrains. In order to compare the effects of each blood-group genotype on mortality in three different populations, namely strain 6 (studied in a previous generation) and substrains 6D and 6F, we required methods of calculating (1) an estimate of the absolute mortality ascribable to a genotype, rather than a relative value; (2) the standard error of this estimate; and (3) an adjustment for those random or specific differences between populations which apply equally to all genotypes. Estimates of absolute mortalities, expressed as *fitted mortalities*, were derived within each population from the relative mortalities of the genotypes, their expected frequencies, and the mean mortality of the population. The rationale for calculation of standard errors was as follows: The standard error of the difference between the mortalities of the ith and jth genotypes within a population may be expressed as $\sqrt{(c_{ii}-2c_{ij}+c_{jj})s^2}$, where s^2 = the estimated error variance, c_{ii} and c_{jj} are coefficients inversely proportional to the number of items of data contributing to the estimates of the mortalities of the ith and jth genotypes, and c_{ij} is a coefficient estimating the extent to which the mortalities of the two genotypes are estimated by the same rather than different items of data. For p genotypes, the sum of the error variances of the $p-1$ differences of the first genotype from each of the others,

$$\sum_{i(j=1)}^{p-1}(c_{ii}-2c_{ij}+c_{jj})\,s^2 = (p-2)\,c_{11}s^2+\sum_{i}^{p}c_{ii}s^2-2\sum_{i}^{p-1}c_{ij}s^2.$$

Since the c_{ij} terms arise because of chance association or dissociation of blood groups within egg-white classes, they tend to sum to zero. Thus the sum of the error variances of all the $p(p-1)/2$ comparisons possible in the population,

$$\sum_{i}^{p-1}\sum_{j}^{p-1}(c_{ii}-2c_{ij}+c_{jj})s^2 = 2(p-1)\sum_{i}^{p}c_{ii}s^2,$$

whence by substitution in the first expression an estimate of the error variance of the first genotype is obtained,

$$c_{11}s^2 = \frac{1}{p-2}\sum_{i(j=1)}^{p-1}(c_{ii}-2c_{ij}+c_{jj})s^2-\frac{1}{2(p-1)}\sum_{i}^{p-1}\sum_{j}^{p-1}(c_{ii}-2c_{ij}+c_{jj})s^2.$$

https://doi.org/10.1017/S0016672300001622 Published online by Cambridge University Press

The adjustment for between-population differences applying equally to all genotypes was calculated as the weighted mean difference between the two sets of genotypic fitted mortalities. The weighting factors were not calculated directly from the genotypic frequencies, since the information on mortality of a genotype was dependent on the form of its distribution among egg-white classes as well as on its raw frequency. Allowance for this was however already made in the estimation of the error variances of the fitted mortalities, so that the reciprocals of these, $(1/c_{ii}s^2)$ were used to calculate weighting factors of the differences.

All computations were performed on the TITAN computer of the University of Cambridge Mathematical Laboratory. The computer programmes were again written for us by Mr Robert Marrs of the ARC Statistics Group, Cambridge, whom we sincerely wish to thank. In the following text and tables the short form *36/38* etc. is used to signify B^{36}/B^{38} etc.

Table 1. *Variance analyses of B blood groups of dams and embryos with total embryonic mortality (TEM)*

	6D				6F			
	D.F.	M.S.	V.R.	*P*	D.F.	M.S.	V.R.	*P*
By dams								
Between blood groups	8	245·52	1·27	—	9	321·87	1·91	< 0·10
Within blood groups	218	192·66			232	168·38		
By embryos								
Between blood groups	8	163·72	< 1	—	9	376·26	2·26	< 0·025
Within blood groups	218	195·66			232	166·27		
By dams and embryos								
Between blood groups	16	192·94	< 1	—	18	284·18	1·72	< 0·05
Within blood groups	210	194·65			223	165·23		

Note: In each analysis, the total of sums of squares is calculated as the sum of squares within dams' egg-white genotypic classes.

4. RESULTS

Pure strains 6D and 6F. Although respectively 639 and 753 young females of substrains 6D and 6F were listed as breeders, only 581 and 582 of them were satisfactorily bloodtyped. Most of the missing birds had died or were culled before bloodtyping was carried out, or had lost their wingbands, or were missed in the sorting; and a few were excluded because of bloodtyping errors, or because they were not also satisfactorily genotyped for the three egg-white loci (see subsequent paper). Following the elimination of hens laying less than ten fertile eggs, and a few which were alone in their egg-white class, the numbers of hens utilized in the analyses were respectively 252 and 264, and they laid totals of 4188 and 4327 fertile eggs.

Variance analyses of blood-group genotypes with Total Embryonic Mortality (TEM) are summarized in Table 1. In 6F, the results are similar to those found previously in 6, the blood-group effects by embryos being significant ($P < 0·025$) and those by dams not ($P < 0·10$). The size of the latter probability indicates possible dam effects. These however cannot be separate and independent of embryo

effects, since if they were the effect of dams and embryos together would have a probability as low or lower than that of embryos alone. This does not occur (P just $< 0{\cdot}05$), so that in 6F as in 6 the blood-group effects are best represented by fitting parameters for the embryo genotypes alone. In 6D there are no significant blood-group effects on TEM. These negative overall findings do not seem to result from a balance between opposing effects in the various periods, as may be seen from the variance analyses by embryos in the partial periods (Table 2). The only period to show significance in 6D is Dead in Shell (DIS). In 6F on the other hand there are indications of effects throughout incubation, with major significant expression in Dead Germs 2 (DG2).

Table 2. *Variance analyses of blood groups of embryos with mortality in the partial periods of incubation*

	6D				6F			
	D.F.	M.S.	V.R.	P	D.F.	M.S.	V.R.	P
First Week Deaths (days 1–7)								
Between blood groups	8	27·37	< 1	—	9	45·31	1·35	—
Within blood groups	218	50·64			232	33·61		
Early Dead Germs (days 1–15)								
Between blood groups	8	49·86	< 1	—	9	97·34	1·71	< 0·10
Within blood groups	218	62·73			232	57·07		
Dead Germs 2 (days 16–20)								
Between blood groups	8	86·89	< 1	—	9	272·93	3·00	< 0·01
Within blood groups	196	139·19			219	90·99		
Dead in Shell (days 20–21)								
Between blood groups	8	93·26	2·44	< 0·025	9	63·76	1·61	< 0·20
Within blood groups	161	38·17			179	39·64		

See footnote to Table 1.

More efficient use of the data is made by testing the significance of all the possible two-way comparisons between genotypes, using Duncan's test. There were 36 comparisons between 9 genotypes in 6D (*37/37* did not occur); and 45 comparisons between 10 genotypes in 6F. For purposes of comparison we also applied Duncan's test to the differences we reported from the ancestor strain 6 four generations previously (Morton *et al.* 1965). Table 3 shows the differences in 6, 6D and 6F which are significant by Duncan's test, with significance levels marked. It should be noted that some significant differences occur in periods which showed no overall significance in the variance analyses (FWD and EDG in 6F; all partial periods in 6). According to Duncan (cited by Balaam, 1963), the detection of some significant differences in cases where there is no overall significance is a valid use of his test. As Winer (1962) pointed out more generally, if several of the contributing means lie close together near the overall mean, it is possible for the overall variance to be non-significant by the F test when a few extreme means are significantly different by a range test. Nevertheless, when interpreting such significant differences, we cautiously paid attention to only those which were independently confirmed in other periods or in the Total Embryonic Mortality. We also

include in Table 3 some other differences (unmarked) which comply with the single t test (D/SE > 2·00, since in all cases ∞ > df > 60) in the period concerned, and are significant by Duncan's test in another. These are regarded as near-significant comparisons, since they also meet the reasonable criterion that effects in the partial periods must relate sensibly to those found in other periods or in Total Embryonic Mortality.

In 6D the only significant differences between genotypes are found in DIS, *36/38* being significantly superior to *35/36* and *35/38*. These differences are valid without further confirmation since they occur in a period with overall significance in the F test. In 6F two genotypes, *35/38* and *37/38*, are markedly inferior in TEM and consistently throughout most of incubation; while two others, *35/36* and *38/38*, are superior in TEM and throughout. *36/38* is rather less inferior in TEM and DG2, while in FWD and EDG it is superior to the poorest genotype *35/38*, so that in general it appears to be intermediate. For *37/37* we may validly conclude that it has by far the highest mortality in DG2, since this period shows overall significance, but this effect does not show in TEM. The remaining four genotypes show significant superiority in the overall non-significant EDG period, but only for *36/36* is this confirmed in TEM. Two of them, *35/35* and *35/37*, are however validly superior to *37/37* in DG2. The differences involving the last genotype, *36/37*, do not meet our criteria for statistical validity.

Our conclusions for strain 6, based on the re-tested comparisons, are slightly modified from those we drew from single t tests in our previous paper. We now find that in TEM two genotypes, *35/36* and *36/36*, were markedly inferior to three superior genotypes, *35/38*, *36/37* and *36/38*, each of these five having at least two significant comparisons. *38/38* was intermediate, having one significant comparison with a superior genotype and one with an inferior. Finally *35/37* and *37/38* were respectively superior or inferior, but with only one significant comparison each. Most of the few differences occurring in the partial periods involved *35/38* and *36/36*, the genotypes with most differences in TEM. In addition, *37/37* had significantly higher mortality in DG2 than *35/38*, but since this was in an overall non-significant period it cannot be accepted in the absence of significant confirmation in TEM. In both 6F and 6 there is no evidence that any blood-group gene is consistently superior or inferior: the associations are with genotypes, not genes.

Comparison of 6F with 6 (Table 3) shows that, of the six genotypes with statistically significant differences in TEM in 6F, five have markedly changed in relative placing. *35/36* and *38/38* are now strongly superior (previously inferior or intermediate respectively); *35/38* is now definitely inferior (previously superior); *36/38* is now inferior or intermediate (previously superior); and *36/36*, previously definitely inferior, is now moderately superior. The sixth, *37/38*, remains inferior as before. The evidence on the other four genotypes is less compelling in one or other generation. *35/37* probably remains superior, at least in some partial periods. *37/37* shows inferiority in DG2 in both generations, which is not demonstrable in either case in TEM, probably because of its rarity. Nothing could be concluded about *35/35* in strain 6, or *36/37* in 6F.

In view of the evidence of changes in relative mortalities between 6 and 6F, we made direct comparisons of genotypic mortalities between populations. Estimates of fitted total embryonic mortalities, their standard errors and adjustments for

Table 3. *Mortality differences (in angles) between blood-group genotypes of embryos in the pure strains 6D, 6F and 6*

6D (Superior genotypes are listed horizontally; inferior vertically)

Dead in Shell

	36/38
35/36	8·4*
35/38	7·3*

6F

First Week Deaths

	36/38	*38/38*	*36/37*	*35/37*
37/38	—	6·4	—	15·2
35/38	5·9*	6·6	8·0	15·3

Early Dead Germs

	36/38	*36/36*	*36/37*	*38/38*	*35/36*	*35/37*	*35/35*
37/38	—	—	—	11·0*	12·2*	21·3*	26·8*
35/38	8·1*	8·5*	10·8*	12·6**	13·8*	22·9*	28·4*

Dead Germs 2

	36/36	*35/36*	*38/38*	*35/37*	*35/35*
36/38	—	11·5	11·7***	—	—
35/38	10·0	15·5*	15·7**	—	—
37/38	—	16·6*	16·8**	—	—
37/37	—	44·2*	44·4*	50·5*	50·6*

Total Embryonic Mortality

	36/38	*36/36*	*36/37*	*38/38*	*35/36*
36/38	—	—	—	12·4*	16·0*
37/38	—	—	—	20·8*	24·3*
35/38	11·1	14·7*	19·2	23·5***	27·1**

6

Early Embryonic Deaths†

	36/38	*35/38*
38/38	—	13·9
36/36	25·8*	28·3*

Dead Germs 1‡

	36/37
36/36	21·8*

Dead Germs 2

	35/38
36/36	18·5
37/37	47·8*

Dead in shell

	36/37
35/36	16·7

Total Embryonic Mortality

	38/38	*36/38*	*35/38*	*35/37*	*36/37*
38/38	—	—	19·0*	—	—
37/38	—	—	20·4*	—	—
35/36	—	30·0*	35·5*	—	39·5*
36/36	26·7*	40·2*	45·7***	47·1*	49·7*
37/37	—	—	53·3	—	—

Probabilities * < 0·05, ** < 0·01, *** < 0·001. † Days 1–5, ‡ days 6–15.

general differences between populations were made as described in Methods. The latter adjustments, calculated as the weighted means of the differences between the fitted mortalities of corresponding genotypes, were 9·1 ± 2·3° for 6 with 6D and

https://doi.org/10.1017/S0016672300001622 Published online by Cambridge University Press

13·5 ± 2·3° for 6 with 6F (or respectively 2·5 % and 5·5 %), both being reductions in mortality, and both significant at $P < 0{\cdot}001$. These differences, due presumably to general effects of year, location and management changes, were added to each fitted mortality in 6D or 6F respectively to make them comparable with those in 6. These *adjusted* fitted mortalities were then used to estimate differences between populations specific to some genotypes, by means of t tests. In these, the error variance of the appropriate adjustment was added to those of the fitted mortalities. Although it was necessary to perform the statistical tests on the transformed angular scale, in presenting the results (Table 4) we have retransformed the fitted mortalities to percent values so that the magnitude of the changes may be more easily appreciated by those unfamiliar with the angular tranformation. Five genotypes show significant differences between 6 and 6F, and these are, as expected, the same five which were noted above as changing in relative placing. The mortality changes include large increases (*35/38*) as well as large decreases (*35/36* and *36/36*). Also in Table 4 are the expected frequencies of the blood-group genotypes among zygotes, based on Mendelian expectation and, as in the calculation of the mortalities, giving equal weight to each mating. Since the frequency of a genotype gives some indication of the likelihood of detecting significant differences, it is noteworthy that in 6 and 6F the rarer genotypes are the only ones not involved in significant differences in TEM (Table 3), and even these (except *35/35* in 6 and *36/37* in 6F) show valid significant differences in one or more partial periods. Thus, with these two exceptions, all genotypes show some evidence of association with embryonic mortality in both 6 and 6F.

By contrast, no significant blood-group effects are shown in TEM in 6D (Table 3) whether of common or rare genotypes. This is confirmed by the mortality values in Table 4, most of which lie within a narrow range, about 43–51 %. Of the three values well outside this range, that of *37/38* is based on a small amount of data and is a poor estimate with large standard error. The other two are those of the most frequent genotypes, *36/38* and *38/38*, and are good estimates with low standard errors. Their differences from the majority of the population are thus at least suggestive of blood-group effects. Comparing between populations, *35/38* has a significantly higher mortality in 6D than in 6 (Table 4). There is also one significant comparison between 6D and 6F, which is not marked in Table 4 because it is based on the separately calculated overall adjustment of 6D with 6F. This is for *36/38*, which has significantly lower mortality in 6D than in 6F ($P < 0{\cdot}05$). Thus although these two genotypes are not involved in significant mortality differences within 6D (except in the partial period DIS, see Table 3), they do show significant between-population differences. In each case their mortality in 6D is intermediate between the values in 6 and 6F. A similar pattern is shown by two other genotypes (Table 4), the mortalities in 6D of both *38/38* (a good estimate) and *35/36* (a fairly good estimate with low standard error, based on 10·1 % of the data) being close to halfway between the values in 6 and 6F.

Crosses 5D × 6D and 5F × 6F. After application of the criteria previously described, respectively 384 and 414 hens of 6D and 6F provided the mortality

data, based on totals of 10392 and 11263 crossbred fertile eggs. The analyses were made by embryo blood groups only, since the results from the dams when bred pure showed no evidence of dam effects independent of embryo effects. Moreover,

Table 4. *Expected zygotic frequencies and fitted total embryonic mortalities (in percents) of blood-group genotypes*

(Comparison of strains 6D and 6F with 6.)

	6		6D		6F	
	Zygotic frequency	Fitted mortality	Zygotic frequency	Fitted mortality†	Zygotic frequency	Fitted mortality†
35/35	0·028	50·7	0·027	47·8	0·011	15·8
35/36	0·059	69·2	0·101	44·3	0·043	21·7*
35/37	0·042	11·0	0·013	49·4	0·017	21·5
35/38	0·162	12·6	0·131	42·8*	0·118	66·9***
36/36	0·044	84·1	0·056	44·7	0·091	41·7*
36/37	0·051	8·4	0·034	51·1	0·046	34·1
36/38	0·209	19·6	0·304	29·8	0·296	47·9**
37/37	0·012	92·6	—	—	0·005	79·2
37/38	0·120	43·5	0·047	23·7	0·095	62·3
38/38	0·273	41·0	0·286	33·8	0·277	27·0*

Probabilities of differences from strain 6: * < 0·05, ** < 0·01, *** < 0·001.
† Adjusted for overall population difference from strain 6; see text.

Table 5. *Variance analyses of blood groups of embryos with mortality in the crossbred populations*

	(5D × 6D)				(5F × 6F)			
	D.F.	M.S.	V.R.	*P*	D.F.	M.S.	V.R.	*P*
First Week Deaths								
Between blood groups	11	38·95	1·54	< 0·20	15	25·17	1·13	—
Within blood groups	348	25·33			372	22·18		
Early Dead Germs								
Between blood groups	11	49·82	1·48	< 0·20	15	22·91	< 1	—
Within blood groups	348	33·77			372	25·72		
Dead Germs 2								
Between blood groups	11	26·84	< 1	—	15	39·85	< 1	—
Within blood groups	341	34·79			371	43·53		
Dead in Shell								
Between blood groups	11	31·45	1·74	< 0·10	15	37·18	1·33	< 0·20
Within blood groups	337	18·08			367	27·97		
Total Embryonic Mortality								
Between blood groups	11	79·00	1·09	—	15	104·90	1·48	< 0·20
Within blood groups	348	72·45			372	70·68		

See footnote to Table 1.

any embryo effects now found would concern a new series of heterozygous genotypes. The variance analyses, given in Table 5, show little evidence of overall blood-group effects in any period. For reasons discussed previously we applied Duncan tests to the two-way comparisons, but used suitable caution in

the interpretation by requiring independent confirmation of effects. There were 16 genotypes in 5F × 6F giving 120 comparisons, but only 12 genotypes and 66 comparisons in 5D × 6D, since the *9* gene was absent from the 5D sires. The results are given in Table 6, which was compiled on the same criteria as Table 3. As might be

Table 6. *Mortality differences (in angles) between blood-group genotypes of embryos in the crossbred populations (5D × 6D) & (5F × 6F)*

(Superior genotypes are listed horizontally; inferior vertically.)

(5D × 6D)

First Week Deaths

	8/38	*10/38*	*8/36*	*7/35*	*7/37*
8/37	—	—	—	—	12·8
10/37	6·9*	7·6*	7·8*	10·8	17·6*

Early Dead Germs

	10/38	*8/38*	*7/37*
8/37	5·7	5·8	16·6*
10/37	—	—	16·9

Dead in Shell

	10/36	*8/38*	*10/38*	*8/35*	*10/37*	*7/36*
8/37	5·3*	5·6*	6·0**	6·1*	9·1*	9·7*
7/35	—	—	—	—	10·3*	—

Total Embryonic Mortality

	8/38	*7/36*	*7/37*
8/37	8·8	15·5*	25·7*

(5F × 6F)

First Week Deaths

	8/35	*9/38*	*9/36*	*8/37*	*7/36*
9/37	17·7*	20·0*	20·1*	20·5*	21·7*

Early Dead Germs

	8/37	*7/36*
8/36	4·9*	—
9/37	16·5	17·8

Total Embryonic Mortality

	8/37
8/38	8·2*
8/36	10·1**
8/35	11·3**
9/37	32·0
7/37	38·5*

Probabilities as in Table 3.

expected from the F tests, relatively few genotypes are involved in comparisons significant by Duncan's test. In 5D × 6D the confirmed effects are the inferiority of *8/37* to *7/37* in one partial period (EDG) and in TEM, and its inferiority to *7/36* in DIS and TEM. In addition, unconfirmed, *8/37* is generally inferior in DIS. In 5F × 6F the only adequately confirmed effect is the superiority of *8/37* over *8/36* in EDG and TEM (the latter at ($P < 0{\cdot}01$), although the superiority of *8/37* over *8/35* in TEM, being at $P < 0{\cdot}01$, perhaps does not require confirmation. *8/37* also shows an unconfirmed but large superiority over *7/37* in TEM, and this in

particular, as well as its other superiorities, is in complete contrast to its position in the other cross. In general it appears that the blood groups are not a very important source of variability in the much reduced mortality of the progeny of these breed-crosses.

5. DISCUSSION

Since it was obviously not possible to bloodtype the embryos which died, we had to devise an indirect method of estimating the mortality associated with each genotypic class of embryos. The mortality ascribable to the embryos laid by each dam was apportioned to the various embryo genotypes she would be expected to produce by her mate, in the proportions expected on normal Mendelian segregation. Parametric estimation of embryo genotypic mortalities was then made as described under *Blood-group analysis*. It is clear that embryo genotype comparisons could not be made within dam-families. We were thus unable to utilize the method of choice for avoiding an important possible source of error, namely that spurious blood-group effects might be generated by confounding with other genetic effects. Although this error is much less likely in analyses by genotypes rather than by genes, it could arise if at least some genotypic classes were largely derived from different sectors of the ancestor population (stratification). Even without stratification, such confounding might conceivably occur randomly if a particular genotypic class were largely derived from a sire or exceptionally prolific dam who happened also to transmit particular non-blood-group effects. This random association would rapidly become less likely as more parents contributed to one genotype. Confounding of either sort would operate particularly through the immediate sires of the individuals studied, and might thus be reinforced by non-genetic pen effects, since there was one sire to each pen. Dams would be less important individually because they usually produce fewer progeny than sires, but could be a source of stratification error if several came from the same grandparents, particularly the grandsire.

Inspection of the derivation of genotypic classes showed that these types of error were not possible in the majority of our analysis. With few exceptions (see below), each class—whether of zygotes or dams—was derived from numerous sires and maternal grandparents constituting a wide sample of families of the present and the previous generations. Putting it another way, almost all sires and maternal grandparents contributed descendants to two or more genotypic classes. Consequently no correction was necessary for error due to family confounding. It is impracticable to present the full mating data here, but a synopsis of some facts relating to zygotes of the pure lines is given in Table 7. This lists the total number of sires, maternal grandsires and maternal grandams of the zygotes entering the analyses, and shows how many of them contributed to each genotypic class of zygotes. Comparison of the latter figures with the totals demonstrates that most genotypes were derived from a considerable proportion of the total breeding population, and thus could not have come exclusively from any genetically distinct group of ancestors, In addition, most genotypes were derived from numerous sires

and thus could not be involved in random sire confounding. These conclusions cannot validly be drawn for certain genotypes (*35/37* in 6D; *35/35*, *35/37* and *37/37* in 6F), which were in fact the rarest (see Table 4). Of these, only *37/37* did come from a single segment of the ancestor population and a single sire, and might thus be subject to both types of confounding error. The other three were each derived from several unrelated sources, so that stratification error was excluded, but random sire confounding was perhaps possible. However, none of these four genotypes was involved in important blood-group findings (see Results), probably because of their rarity, and so the question of error does not arise for them.

Table 7. *Total number of sires and maternal grandsires and grandams of embryos entering the 6D and 6F analyses, and number of each contributing to each embryo genotype*

	Total number	Number contributing to each embryo genotype									
		35/35	*35/36*	*35/37*	*35/38*	*36/36*	*36/37*	*36/38*	*37/37*	*37/38*	*38/38*
6D											
Sires	46	9	22	3	28	17	9	38	0	11	37
Maternal grandsires	18	6	11	2	14	10	6	17	0	8	17
Maternal grandams	95	14	36	5	45	26	12	77	0	19	63
6F											
Sires	46		10	6	20	17	15	40	1	20	40
Maternal grandsires	23	4	7	5	14	11	13	21	1	15	22
Maternal grandams	96	5	15	6	31	24	19	70	2	32	78

The conclusion above, that family confounding could not be a source of error, is confirmed by some of the results. For example, if confounding with dam's or dam's family's genetic contribution were to generate spurious blood-group effects, such confounding would necessarily be greater in the dam than in the progeny analysis, and the analysis by dam blood groups would account for the larger part of the variance. This was not so (Table 1). Furthermore, family confounding, particularly via sires, would more readily generate gene than genotype effects in the embryos, which is the reverse of our finding (Table 3).

We have confirmed an association of *B* blood-group genotypes of embryos with mortality during incubation; although directly in only one of the two substrains descended from the strain 6 birds studied previously (Morton *et al.* 1965). An important new finding, from the comparison of 6F with 6, is that the mortalities associated with five out of ten genotypes have changed markedly. This is seen both from the comparisons between different genotypes in Table 3, and from the comparisons of the same genotypes between different populations in Table 4. In the latter, we first corrected for the significant decrease in mortality in the population as a whole over the 4 years between, calculated as the decrease in mortality averaged over all genotypes. Three genotypes have decreased in mortality significantly more than this average, while two have actually increased. In these five there has thus been a definite genotype–environment interaction, either directly between the changing environment and the *B* blood groups or via other parts of

the genome which themselves interact epistatically with the blood-group genotypes. This evidence of specific changes from 6 to 6F concerns only the five genotypes for which we have the best statistical information, and the data are inadequate for any conclusions on the other five genotypes. The evidence is thus in favour of change rather than stability. For 6D we have no within-population evidence, but there is some information on changes. The four mortalities of which we have good estimates in 6D are of genotypes showing marked changes between 6 and 6F, and in every case are about half way between the extreme values found in these. This suggests that the processes causing specific changes between generations have applied to a lesser degree to 6D, leading to intermediate genotypic mortalities which do not differ from one another within 6D sufficiently for effects to be demonstrable. It is obvious that the findings are equally consistent with the view that *B* blood groups have no effect on mortality in 6D, but this seems unreasonable in view of the marked effects in both the sister strain 6F and the ancestor strain 6. We may suppose that changes in environmental conditions such as housing, management of the birds, incubator routine etc. were less from 6 to 6D than from 6 to 6F; while differentiation in the rest of the gene complex may have played a part.

We are unable to demonstrate whether the blood-group genotypic associations with mortality are due to pleiotropic effects of the blood-group genes themselves, or to effects of genes at a linked locus which themselves affect mortality. If the latter, then our results can be reasonably explained only if such a locus also has four alleles which show the same specific genotype-associations as those we have demonstrated. We would then be considering short chromosomal segments or 'effective factors' (Mather, 1949) rather than genes at either locus. The linkage alternative might be important if we could thereby explain the reversal of the selective forces on certain *B* genotypes from 6 to 6F. Even if such an interpretation could be accommodated to the complexity of the observed genotype-associations, it would still require that a large segment of the present 6F population derived from a single strain 6 or intervening ancestor in whom a crossover occurred. This is not compatible with the known population structure. Alternatively, a linkage explanation would require the highly unlikely event that the same crossover, or several different crossovers, occurred many times in the intervening period. We thus believe that our findings involve either the *B* locus itself, or the very short chromosomal segments including the *B* locus, and we shall use *B* as descriptive of either.

The finding of marked changes between 6 and 6F, with 6D possibly intermediate, requires us to change our previous views (Morton *et al.* 1965) on the effect of *B* on hatchability. We concluded that there seemed to be general overdominance in strain 6, although the genotypic mortalities could not alone account for a stable polymorphic equilibrium of the four gene frequencies, but would lead to a polymorphism of *35* and *38* only. We therefore presumed the existence of balancing selective forces in later parts of the life cycle. It is now clear that no satisfactory conclusions can be drawn from the situation in one generation, since we have shown

that the selective pressures on the *B* locus from differential embryonic mortality vary from generation to generation and even between populations differing apparently only slightly in environment and genetic composition.

Varying selection pressures were proposed as one mechanism for stable polymorphism by Haldane & Jayakar (1963). They considered only the two-allele case, and showed mathematically that polymorphism would persist if the long-term fitness of each homozygote, calculated as the geometric mean of the fitnesses in a number of generations, were less than that of the heterozygote similarly calculated. In practical terms this might occur if the homozygotes were usually fitter than the heterozygote, but were occasionally much less fit. We have not attempted the formal extension of their criterion to the multiple allele case, but we suppose that a combination of it with the criteria of Mandel (1959) would apply. We have calculated the geometric means of the percent embryonic viabilities of the genotypes over each of the double combinations of generations or substrains, and in the triple combination. With one exception, the values fit the two simplest of Mandel's criteria: the geometric mean viabilities of most heterozygotes exceed those of most homozygotes, and those of three of the homozygotes are below the geometric means of population means. The exception is *38/38*, whose geometric mean viabilities are in some but not all combinations superior to those of its heterozygotes (usually not *36/38*), and those of the population means. We would thus not expect the polymorphism of all four alleles to persist. The likely outcome may be calculated on the assumption that the three sets of mortalities would succeed one another in regular oscillation in future generations (with the value from 6F inserted for the missing mortality value of *37/37* in 6D). On this basis the *35* and *37* genes would eventually be lost, and *38* and *36* would continue in balanced polymorphism, with the frequency of the latter oscillating between 0·14 and 0·16. This calculation is unrealistic, since our results may not be a sufficient sample of all the possible future effects. Nevertheless, the embryonic mortalities in the three samples of this population of the Light Sussex breed do not alone account for maintenance of the polymorphism of four alleles. We may now postulate compensatory selective forces not only in other parts of the life cycle, but also in other generations.

We have provided further support for the hypothesis mentioned in the Introduction by demonstrating strong selection on a polymorphic locus with multiple alleles in a relatively noninbred population. Our findings, although incomplete, are more informative about the selective forces acting upon such polymorphisms in natural populations than are the findings of strong overdominance in inbred lines (Gilmour, 1960; Briles & Allen, 1961). As pointed out in detail previously (Gilmour, 1962; Allen & Gilmour, 1962), such evidence is not very relevant to natural conditions. Inbreeding not only reduces the number of multiple alleles, so precluding varying selective effects on numerous *B* genotypes as seen in our results, but also breaks up the coadaptation of segregating loci which is a feature of outbreeding populations. It is probable that both these changes contribute to the marked overdominance shown by two (or rarely three) surviving *B* alleles for aspects of viability and reproductive performance. The likelihood of epistatic interactions between

polymorphic loci will be further discussed in a subsequent paper on the egg-white loci.

Our success in showing strong selective effects contrasts with the failure of other investigations of polymorphisms and selective factors in relatively noninbred populations. Extremely few associations were found out of very large numbers of tests made in cattle by Neimann-Sørensen & Robertson (1961), in sheep by Stansfield *et al.* (1964) and in man by Morton, Krieger & Mi (1966). In the last, the only effects found involved maternal/foetal incompatiblity in the ABO system, and these are not of comparative interest to us. We shall discuss diallelic loci separately in a subsequent paper. For polyallelic loci, we suggest that the lack of results stems from the fact that all these authors tested the association of serological factors or genes, rather than genotypes, with selective effects, on the assumption that the genes act additively via pleiotropy or linkage. In a few cases, they also tested for overall heterozygote advantage by comparing mixed groups of heterozygotes and homozygotes. In examining why the *B* locus in chickens continues in stable polymorphism, as it undoubtedly does (Gilmour, 1960), we were influenced by arguments of Mandel (1959) and one of us (Gilmour, 1960, 1962) to expect that differences in selective values of the various heterozygotes at a polyallelic locus would be more marked than any overall heterozygote advantage. Accordingly, we utilized a parametric analysis giving relative mortalities of all the individual genotypes, and found genotype-associations which changed between generations and environments. Had we tested only average effects of genes, some results might have been found, such as the slight superiority of *38* over the three populations; but very poor use would have been made of the available information.

REFERENCES

Allen, C. P. & Gilmour, D. G. (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. Princeton* **47**, 1711–1718.

Balaam, L. N. (1963). Multiple Comparisons—a sampling experiment. *Australian Journal of Statistics* **5**, 62–84.

Briles, W. E. & Allen, C. P. (1961). The B blood group system of chickens. II. The effects of genotype on livability and egg production in seven commercial inbred lines. *Genetics. Princeton* **46**, 1273–1293.

Cochran, W. G. (1943). Analysis of variance for percentages based on unequal numbers. *Journal of the American Statistical Association* **38**, 287–301.

Duncan, D. B. (1955). Multiple range and multiple *F* tests. *Biometrics* **11**, 1–42.

Duncan, D. B. (1957). Multiple range tests for correlated and heteroscedastic means. *Biometrics* **13**, 164–176.

Gilmour, D. G. (1960). Blood groups in chickens. *British Poultry Science* **1**, 75–100.

Gilmour, D. G. (1962). Current status of blood groups in chickens. *Annals of the New York Academy of Sciences* **97**, 166–172.

Haldane, J. B. S. & Jayakar, S. D. (1963). Polymorphism due to selection of varying direction. *Journal of Genetics* **58**, 237–242.

Harter, H. L. (1960). Critical values for Duncan's new multiple range test. *Biometrics* **16**, 671–685.

Kramer, C. Y. (1956). Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics* **12**, 307–310.

KRAMER, C. Y. (1957). Extension of multiple range tests to group correlated adjusted means. *Biometrics* **13**, 13–18.

LUSH, I. E. (1961). Genetic polymorphisms in the egg albumen proteins of the domestic fowl. *Nature, London* **189**, 981–984.

LUSH, I. E. (1964). Egg albumen polymorphisms in the fowl: loci *II* and *III*. *Genetical Research* **5**, 39–49.

MANDEL, S. P. H. (1959). The stability of a multiple allelic system. *Heredity. London* **13**, 289–302.

MATHER, K. (1949). *Biometrical Genetics*. London: Methuen.

MORTON, J. R., GILMOUR, D. G., MCDERMID, E. M. & OGDEN, A. L. (1965). Association of blood-group and protein polymorphisms with embryonic mortality in the chicken. *Genetics. Princeton* **51**, 97–107.

MORTON, N. E., KRIEGER, H. & MI, M. P. (1966). Natural selection on polymorphisms in Northeastern Brazil. *American Journal of Human Genetics* **18**, 153–171.

NEIMANN-SØRENSEN, A. & ROBERTSON, A. (1961). The association between blood groups and several production characteristics in three Danish cattle breeds. *Acta agriculturae Scandinavica*. **11**, 163–196.

OGDEN, A. L., MORTON, J. R., GILMOUR, D. G. & MCDERMID, E. M. (1962). Inherited variants in the transferrins and conalbumins of the chicken. *Nature, London* **195**, 1026–1028.

STANSFIELD, W. D., BRADFORD, G. E., STORMONT, C. & BLACKWELL, R. L. (1964). Blood groups and their associations with production and reproduction in sheep. *Genetics. Princeton* **50**, 1357–1367.

WINER, B. J. (1962). *Statistical Principles in Experimental Design*. New York: McGraw-Hill.