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Association of Genetic Polymorphisms with Embryonic Mortality in the Chicken

III. Interactions between Three Loci Determining Egg-white Proteins

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Summary. Association of the egg-white genotypes of dams with the mortality of their embryos was studied in 1966 in the pure and crossbred embryos of dams of two relatively noninbred Light Sussex substrains, 6D and 6F. These had been derived two generations earlier, in 1964, by equal division of strain 6, which we previously studied in 1962; while the sires of the crosses came from two Rhode Island Red/New Hampshire substrains, 5D and 5F, similarly derived. The effects were estimated parametrically for the three loci *Tf*, *II* and *III* singly and in two- and three-way interaction.

In the first week of incubation, *Tf* had an additive effect in all matings, while *II* (and perhaps *III*) had a near-additive effect in the pure matings only. There was an additive × additive interaction effect of *II* × *III* in the last six days of incubation, which was consistent over all matings except 5F × 6F. The only effect to influence the total mortality over the whole of incubation was the three-way additive × additive × additive interaction, and there was substantial agreement in this over the matings. Some triple homozygotes were superior and others inferior to the genotypes with one or more loci heterozygous.

This evidence of multiple interaction between loci affecting fitness is discussed in relation to hypotheses for the maintenance of genetic variability in populations.

Introduction

In a previous paper (Gilmour and Morton, 1970) we gave our results on the association of genotypes at the polyallelic *B* blood-group locus with embryonic mortality in pure and cross progeny of two relatively noninbred substrains of Light Sussex chickens, and compared them with our original findings in the ancestor strain (Morton, Gilmour, McDermid, and Ogden, 1965). Taken together, the results led us to the conclusion that varying selection pressures, between generations and environments, may be of great importance in the maintenance of stable polymorphism of multiple alleles or linked combinations.

In this paper we present the corresponding results and comparison for three diallelic egg-white loci. As we anticipated in the Introductions to the two previous papers in the series, cited above, the epistatic interactions between the loci are more readily demonstrable than effects of the individual diallelic loci.

Materials and Methods

The data consist of the mortalities of embryos from both pure and crossbred matings of dams of two related substrains of Light Sussex chickens. Details of the history and maintenance of the chickens and of the organization of data were given by Gilmour and Morton (1970). As before, the pure matings of the two Light Sussex substrains are referred to as 6D and 6F; and the cross matings of the same dams to males of two Rhode Island Red/New Hampshire substrains as 5D × 6D and 5F × 6F. As before, each embryonic death was assigned to a particular day of the 21-day incubation period by visual inspection of the broken-open egg. In addition to the Total Embryonic Mortality (TEM, days 1–21), calculated as a fraction of the number of fertile eggs, the three distinct periods of high mortality were also analysed. These were First Week Deaths (FWD, days 1–7), Dead Germs Two (DG2, days 16–$20^1/_2$) and Dead in Shell (DIS, days $20^1/_2$–21). The middle low-mortality period (days 8–15) could not be analysed because many dams had zero embryonic mortality; instead, the whole period of days 1–15 was also analysed as Early Dead Germs (EDG). In the partial periods the mortalities were expressed as fractions of the number of embryos at risk at the beginning of each period. For statistical analysis, the fractional mortality values were converted to angular values (in degrees) by the arc-sine transformation.

Egg-white loci: The three co-dominant diallelic loci studied were *Tf*, *II* and *III*. All three loci were segregating in 6D and 6F in polymorphic frequencies, in that the lowest frequency of any single genotype among the dams was >0.05 (0.054 for Tf^a/Tf^a in 6F). *Tf* determines variation in conalbumin of the egg white, and parallel variation in serum transferrin (Ogden, Morton, Gilmour and McDermid, 1962), since conalbumin and transferrin are both glycoproteins with identical amino-acid sequences but differing carbohydrate prosthetic groups (Williams, 1962). *II* and *III* determine variation in two other egg-white proteins (Lush, 1961, 1964a) which are loosely called 'globulins' although they have not yet been precisely characterized. At each of the three loci the alleles *a* or *b* determine patterns 'a' or 'b', which run respectively fast or slow in starch-gel electrophoresis in alkaline conditions, with both appearing in the heterozygote. Phenotypes and hence genotypes for the 6D and 6F dams were thus directly determined by subjecting their egg white to the starch-gel electrophoretic technique described previously (Morton *et al.*, 1965), as used then in the Stock laboratory. This work was carried out for us

in the research laboratory of Thornber Brothers Ltd., Mytholmroyd, Halifax, Yorkshire, under the direction of Mr. E. M. McDermid with the assistance of Mr. A. L. Ogden, to both of whom we are greatly indebted.

Choice of analytical method: For reasons given previously (Gilmour and Morton, 1970), we ignored any interaction between *B* blood groups and egg whites, and analysed by dams' egg-white genotypes only. Although sire genotypes for *Tf* could have been determined by electrophoresis of serum samples, we did not make any other analyses. Loci *II* and *III* presumably have their major action on the composition of egg white, and hence possibly on embryonic mortality; and are unlikely to have important expression in the embryo. In considering the three loci together, and looking for interactions, we were therefore more interested in the expression of *Tf* in the dam, on conalbumin in egg white, than its expression in the zygote, on serum transferrin.

Adjustment for blood-group effects: The embryonic mortality values ascribable to dams are also affected by the *B* blood-group genotypes of their progeny in cases where blood-group effects occur. Since the blood groups are not distributed proportionately among the egg-white types, it is necessary in such cases to adjust the data by the estimated mortality differentials between the blood-group genotypes (Gilmour and Morton, 1970) before proceeding to the egg-white analysis. Experience showed that no error was introduced by also making such adjustment in cases where no significant blood-group effects were demonstrated. All results are therefore from analyses of adjusted data.

Egg white analysis: The three egg-white loci were treated as fixed variables in a three-way cross-classification analysis of variance, with the three genotypes at each locus as the potential 'levels' of each variable. Since we wished to test for genetic interactions between the loci as well as main effects, it was necessary to use the model

$$y_{ijkm} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \alpha\beta\gamma_{ijk} + \varepsilon_{ijkm}$$

where y_{ijkm} is the mortality of embryos from the mth dam with the *ith, jth* and *kth* genotypes at the three loci, while the symbols on the right hand side represent parameters, μ for the general mean; α_i, β_j and γ_k for the main effects of these genotypes; $\alpha\beta_{ij}$, $\alpha\gamma_{ik}$, $\beta\gamma_{jk}$ and $\alpha\beta\gamma_{ijk}$ for the statistical interactions between them, and ε_{ijkm} for the random error.

Since the data were markedly nonorthogonal, it was necessary to use a least-squares method of analysis. In this standard method, each parameter in the model would require one least-squares equation, in each of which an observed mean subclass mortality is equated to the parameters weighted by the appropriate frequencies. In the present case 64 such equations would be required to fit the model, made up of 1 for the mean, 3×3 for the single loci, 3×3^2 for pairs of loci, and 1×3^3 for all three together. No unique solution to these equations is possible, however, until they are reduced in number to the number of subclasses in the data, that is, the number of genotypic subclasses of dams ($= 3 \times 3 \times 3 = 27$).

This reduction was achieved by the usual procedure of imposing the constraints that the estimates of the parameters for the main effects sum to zero within each set, and those for the interactions sum to zero separately over each row, column and 'layer' of the data, as appropriate. Effectively, this involves estimating 2 of the 3 parameters in each main-effect set relative to the third, which is eliminated. Similarly, for the interactions 2^n out of 3^n parameters are estimated relative to the remainder, which are eliminated.

In order to simplify the genetic interpretation of the statistical findings, we chose to eliminate the parameters for heterozygotes. The two parameters estimated for each single locus were thus for the mortality of each homozygote relative to that of the heterozygote. In each two-locus set there were four parameters, for the mortality of each of the four double homozygotes relative to that of the double heterozygote, after allowing for the effects of the appropriate single homozygotes. In genetic terms these latter parameters thus provided estimates of the epistatic interaction between pairs of loci. Similarly there were eight parameters for the mortalities of the eight triple homozygotes relative to the triple heterozygote, after allowing for the effects of the appropriate double and single homozygotes, and these provided estimates of the three-way epistasis.

Application of the constraints required numerous subtractions and additions to be made among the 64 equations such as to reduce them to 27. Detailed procedures for doing this were readily derived by extension from the clear account for the two-factor case given by Harvey (1960). The 27 equations were then solved by matrix arithmetic and the variance due to fitting the parameters calculated by the usual methods. The difference between this variance and that due to fitting any less complete set of parameters was the variance attributable to the set of parameters omitted. Thus variances were derived for the overall effects of each locus (with 2 degrees of freedom), of each two-way interaction (4 df), and of the three-way interaction (8 df), a total of 26 df. Since each varian-

Table 1. *Sample analysis of variance of dam egg-white genotypes with embryonic mortality for one population in one period, and fitted parameters for relative embryonic mortalities (in angles)*

Population: 5F × 6F Period: FWD (days 1 – 7)

	Analysis of variance				Parameters for dams homozygous for					
	d. f.	M. S.	V. R.	*P*	*a*	*b*	*a, a*	*a, b**	*b, a*	*b, b*
Tf	2	84.11	3.79	<.05	−1.2	+1.3				
II	2	25.04	1.13	—	+0.7	−0.8				
III	2	15.09	<1	—	−0.8	+0.3				
Tf × *II*	4	7.27	<1	—			+0.8	−0.6	−0.6	+0.6
Tf × *III*	4	13.91	<1	—			−0.2	+1.2	−0.3	−0.5
II × *III*	4	13.07	<1	—			−1.0	+1.4	−0.0	−0.1
Tf × *II* × *III***	8	17.29	<1	—						
Error	372	22.18								

The error is the mean square within egg-white genotypes within *B* blood-group genotypes.

* Denotes homozygote for *a* at the first-named locus and for *b* at the second.

** For values of fitted parameters, see Table 4.

ce was estimated independently of all others, they could all be tested for significance against the residual variance (within egg-white genotypes within *B* blood-group genotypes).

The matrix solution also provided estimates of the 26 relative mortality parameters. Where there was evidence for an overall effect, study of the values of the parameters allowed distinction to be made between additive and various forms of non-additive genetic 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 interaction involving at least one heterozygote. Significance tests were not applied to the parameters, since these would not add useful information to that already available from the *F* tests of overall effects.

Combination of data: In some analyses, data were combined from different populations (that is, substrains and types of mating) with different mean mortalities. Because of the form of the analysis, the base datum in each population was the mortality ascribable to the triple heterozygote. Before combining data, a fixed value was added to the mortality ascribable to each dam in one population, so as to make the mean mortality ascribable to the triple heterozygote the same in both.

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 Agricultural Research Council Statistics Group, Cambridge, whom we wish sincerely to thank.

Results

Since we were analysing by dam genotypes at three loci whose primary expression was in composition of the egg white, there were grounds for supposing that any effects on embryonic mortality might be seen throughout the whole dam population. On the other hand, the possibilities had to be considered that the effects might be different in crossbred as compared with purebred matings, or in one substrain as compared with another. We therefore made analyses of all the possible logical groupings which might allow investigation of the two types of difference, as well as an analysis of the whole population. The data came from the 6D, 6F, 5D × 6D and 5F × 6F matings, based on totals of 4213, 4351, 10428 and 11285 fertile eggs laid by respectively 254, 266, 385 and 415 hens. Separate analyses of the data from these populations (Table 2, discussed below) allowed preli-

Table 2. *Estimated relative embryonic mortality parameters (in angles) of dam egg-white homozygotes in pure and crossbred matings of substrains 6D and 6F, and the probability of the variance associated with each set of parameters*

Homozygote for			*FWD* (days 1—7)				DG2 (days 16—20)				DIS (days 20—21)				TEM (days 1—21)			
Tf	*II*	*III*	6D	6F	5D × 6D	5F × 6F	6D	6F	5D × 6D	5F × 6F	6D	6F	5D × 6D	5F × 6F	6D	6F	5D × 6D	5F × 6F
b	.	.	+1.4	+0.8	+0.7	+1.3	+0.3	+0.6	+0.9	−1.2	−1.3	−1.1	+0.2	−0.5	−0.2	+0.0	+1.6	−0.5
a	.	.	−0.8	−1.7	−0.5	−1.2	+1.1	+0.2	−1.7	+0.8	+1.3	+2.5	−0.7	−0.1	+1.7	+0.7	−1.9	−0.5
		P	<.20	—	—*	<.05	—	—	—	<.20	—	—	—	—	—	—	—	—
	b	.	−0.1	−1.2	+0.3	−0.8	−0.5	+1.0	+1.2	−0.4	+1.1	−1.2	−0.5	+0.4	+0.1	−0.6	+0.9	−0.6
	a	.	+1.5	+1.7	−0.2	+0.7	+1.6	+1.6	−0.7	−1.5	+0.8	−0.2	+0.6	+0.3	+2.3	+1.2	−0.4	−0.4
		P	<.20	—	—	—	—	—	—	<.20	<.20	—	—	—	—	—	—	—
		b	+1.4	+1.9	−0.7	+0.3	+0.9	+5.0	−0.5	−2.2	+1.8	−1.0	−0.6	+0.8	+2.2	+3.0	−1.1	−0.8
		a	−1.2	−3.0	+0.4	−0.8	−0.4	−4.0	−0.0	+1.8	−1.2	+1.3	+0.0	−0.2	−1.9	−3.1	+0.0	+0.5
		P	—	—	—	—	—	<.10	—	<.05	<.20	—	—	—	—	—	—	—
b	*b*	.	−1.6	−1.2	+0.8	+0.6	+2.0	−0.4	+1.4	+1.1	−1.8	+0.9	+0.4	−0.3	+0.2	−0.6	+2.5	+0.5
b	*a*	.	+0.0	−0.3	−0.6	−0.6	−1.1	+0.5	−1.0	+0.3	+0.1	−0.5	−0.6	−0.5	−0.4	+0.5	−2.0	+0.3
a	*b*	.	−0.1	+0.5	+0.1	−0.6	−1.7	−0.2	−1.0	−0.4	+3.3	−1.5	−1.2	+0.4	−0.5	+0.2	−2.0	+0.4
a	*a*	.	+1.7	+1.0	−0.1	+0.8	+1.4	+1.3	+1.0	−2.3	−1.6	+0.5	+1.0	+0.5	+0.8	−0.1	+2.0	−1.7
		P	<.20	—	—	—	—	—	—	—	<.20	—	—	—	—	—	<.20	—
b		*b*	−0.5	+0.5	−0.7	−0.5	+2.4	−1.4	+0.3	+0.5	+0.8	+0.7	+0.7	−0.8	+2.4	+0.0	−0.1	−0.5
b		*a*	+0.3	+1.4	+0.7	−0.3	−2.2	+1.3	+1.2	−0.8	−1.0	−1.4	−0.4	+1.3	−2.9	+0.1	+1.0	−0.2
a		*b*	+0.9	+0.0	+0.6	+1.2	−1.2	+5.0	−0.8	−2.0	+0.1	−2.0	−1.0	+0.9	−0.8	+1.4	−0.3	+0.6
a		*a*	−0.2	−3.0	−0.6	−0.2	+0.7	−4.2	−0.2	+1.7	+0.3	+2.3	+0.4	−1.3	+1.3	−2.3	−0.3	−0.3
	b		—	—	—	—	—	—	<.20	—	—	—	—	—	—	—	—	—
	b	*b*	−0.3	−0.6	−0.6	−0.1	−0.4	+3.5	+2.1	−0.2	+3.8	+0.5	−0.4	−2.3	+2.3	+3.3	+0.7	−1.8
	b	*a*	+0.1	+0.5	+0.2	−0.0	−1.6	−0.4	−1.3	−1.8	−2.9	−2.1	+0.0	+1.1	−3.0	−0.9	−1.3	−0.8
	a	*b*	+2.2	+0.0	+0.1	+1.4	−0.6	−0.4	−0.4	+0.8	−1.2	−1.3	−0.2	+1.9	+0.1	−1.7	−0.8	+3.1
	a	*a*	−2.0	+0.7	−0.1	−1.0	+4.0	−0.5	+0.4	−0.4	+1.2	+0.7	+0.3	−0.9	+1.7	+0.1	+1.5	−1.6
		P	—	—	—	—	<.20	<.20	<.10	<.20	<.10	<.20	—	—	—	—	—	—

For three-way interaction parameters and probabilities, see Table 4. — * *P* value and parameters for EDG (days 1—15) are: $P < .20$; $b + 1.1$; $a - 0.7$.

minary examination of the various possibilities. When assembling larger groupings from the above data, we first made adjustments (see Materials and Methods) for overall mortality differences between the smaller populations. These were necessary so as to remove confounding sources of variation within the larger groupings, due to general environmental and genetic influences as distinct from the specific genetic influences under study.

A sample analysis of variance of dam egg-white genotypes with mortality of their embryos is given in Table 1. This also shows the least-squares estimates of the parameters for the embryonic mortality associated with each dam homozygous class relative to that associated with the appropriate heterozygous class. In this example, only one of the seven overall effects shows conventional significance ($P < 0.05$) in the F test, namely, the main effect of locus *Tf*. From the values of the parameters it can be decided that this effect is genetically additive, since the *a* homozygote is superior in fitness to the heterozygote (that is, has less embryonic mortality) by almost exactly the same amount as the *b* homozygote is inferior to the heterozygote.

Analyses like the example just described were made of the four populations 6D, 6F, 5D × 6D and 5F × 6F in all five periods of incubation, and are summarized in Table 2. In addition to the parameter estimates, the probability levels for those overall effects approaching significance ($P < 0.10$) are listed, as well as trends ($P < 0.20$). The values for the period EDG (days 1—15) are not given, however, since with one exception (* in Table 2), the significance level for EDG was always lower than for FWD (days 1—7), indicating no separate effect in days 8—15.

None of the seven genetic effects tested in these preliminary analyses shows an excess of significant variance ratios over the number to be expected by chance alone in such a number of tests. Some effects in some periods do however show a cluster of probabilities at < 0.20 or less, accompanied in some cases by agreement in the signs of the parameters. Thus, in all four populations the *a* homozygote at the *Tf* locus is superior to the *b* homozygote over days 1—7, and the double homozygote *b*, *a* at *II*, *III* is superior over days 16—20. Some other agreements include the whole pattern of parameters at *II*, *III* over days 20—21 in 6D and 6F, and (in Table 4, discussed later) a cluster of low probabilities for the three-way interaction late in incubation. There is also agreement in the signs of the parameters at both *II* and *III* over days 1—7 in three out of four populations.

Several of the effects just mentioned show significance in the results of the analyses of all matings combined (ALL, Table 3). Also given in this table are

Table 3. *Estimated relative embryonic mortality parameters (in angles) of dam egg-white homozygotes in all matings, associated with each*

Homozygote for				FWD (days 1—7)					DG2 (days 16—20)				
Tf	*II*	*III*		ALL	6D + 6F	5D × 6F + 5F × 6F	6D + 5D × 6D	6F + 5F × 6F	ALL	6D + 6F	5D × 6D + 5F × 6F	6D + 5D × 6D	6F + 5F × 6F
b	.	.		+0.9	+0.8	+1.1	+0.9	+0.8	−0.1	+0.2	−0.1	−0.1	−1.1
a	.	.		−0.8	−0.7	−1.1	−0.5	−0.8	+0.7	+2.2	−0.7	+0.7	+1.4
			P	<.01	—	<.01	∼.05	—	—	<.05	—	—	—
.	*b*	.		−0.4	−0.9	−0.1	+0.4	−1.1	+0.7	+0.7	+0.5	+0.5	+0.2
.	*a*	.		+0.8	+1.8	+0.2	+0.5	+0.9	−0.1	+1.3	−1.1	−0.2	−1.5
			P	∼.05	<.01	—	—	<.10	—	—	<.10	—	—
.	.	*b*		+0.3	+1.5	−0.4	+0.4	+0.7	+0.6	+2.4	−0.8	+1.0	−0.6
.	.	*a*		−0.6	−1.3	−0.1	−0.4	−1.2	−0.6	−1.2	−0.2	−0.8	+0.7
			P	—	<.05	—	—	—	—	<.10	—	—	—
b	*b*	.		−0.0	−1.1	+0.7	−0.4	+0.2	+0.6	+0.3	+1.2	+1.6	+0.3
b	*a*	.		−0.6	−0.1	−0.8	−0.4	−0.4	−0.2	+0.3	−0.3	−0.7	+1.4
a	*b*	.		+0.1	−0.2	+0.1	+0.6	−0.5	+0.0	−0.6	−0.1	−0.9	+0.4
a	*a*	.		+0.7	+1.2	+0.3	+0.6	+0.6	−0.1	+1.2	−0.9	+0.1	−3.1
			P	—	<.10	—	—	—	—	—	<.05	—	—
b	.	*b*		−0.2	+0.4	−0.5	−0.8	+0.1	+0.1	+1.6	−0.1	+0.3	+0.2
b	.	*a*		+0.2	+0.1	+0.1	+0.6	+0.0	+0.3	−1.4	+1.2	+0.4	−0.7
a	.	*b*		+0.3	−0.1	+0.6	+1.0	+0.5	+0.3	−0.4	−0.3	+0.5	−0.8
a	.	*a*		−0.2	−0.4	−0.0	−0.4	−0.7	−0.5	+1.1	−1.2	−1.0	+1.2
			P	—	—	—	—	—	—	—	<.10	—	—
.	*b*	*b*		−0.3	−0.6	−0.1	+0.1	−0.2	+1.2	+1.9	+0.1	+0.6	+2.1
.	*b*	*a*		+0.3	+0.2	+0.3	−0.0	+0.1	−1.5	−1.2	−1.1	−1.8	−1.8
.	*a*	*b*		+0.7	+1.7	+0.3	+0.8	+1.1	−1.1	−0.5	−0.5	−1.6	−0.4
.	*a*	*a*		−0.6	−1.1	−0.3	−0.8	−0.9	+1.7	+1.8	+1.0	+3.1	−1.0
			P	—	—	—	—	—	<.10	—	—	<.05	—

For three-way interaction parameters and probabilities, see Table 4.

the results for the logical double combinations of populations, namely, both purebreds; both crossbreds; all matings of 6D dams (pure + cross); and all matings of 6F dams (pure + cross). In this table the trends at $P < 0.20$ are not marked, but a few values just outside the cutoff points are marked as $P \sim 0.10$ ($0.099 < P < 0.105$) and $P \sim 0.05$ ($0.0499 < P < 0.0525$). Results for the period EDG are omitted, for the same reason as in Table 2.

The most marked effect in all matings combined is of the *Tf* locus over days 1—7, at $P < 0.01$, with the *a* homozygote superior and the *b* homozygote inferior. Since the estimated relative parameters are nearly equal (−0.83, +0.87), we judge this effect to be genetically additive. The close agreement between trends in the crossbred matings (Table 2) is reflected in the marked effect of *Tf* in their combination (Table 3) at $P < 0.01$. Again, the effect is additive (parameters −1.11, +1.05). The direction of the effect is the same in the purebreds (6D + 6F) as in the crosses, and this close agreement clearly leads to the high significance level ($P < 0.01$) seen in all matings combined. There is thus no indication of pure *versus* cross differentiation, even though no significance occurs in 6D + 6F. This additive effect probably does not continue beyond the first week of incubation, since the significance levels for EDG (days 1—15) are considerably lower. Later in incubation there are some indications of a reversal of this effect. Thus, the *a* homozygote is inferior over days 16—20 in 6D + 6F ($P < 0.05$), and the *a* homozygote tends to be inferior and the *b* homozygote superior over days 20—21 in all matings ($P < 0.10$, ALL, Table 3).

The other significant effect in ALL is of the three-way interaction. Before considering this, we shall present some findings which are near-significant in ALL and significant in some smaller groupings (Table 3). Of these, the most marked in all matings combined is of the *II* locus in the first week of incubation at $P \sim 0.05$. This is a lower significance level than that seen in the pures ($P < 0.01$, 6D + 6F, Table 3). From the significance tests in the other combined populations (Table 3), as well as the indications in Table 2, it is clear that the cross 5F × 6F shows the same effect as the pures, while 5D × 6D does not. The effect, at its highest significance level, in 6D + 6F, is a superiority of the *b* over the *a* homozygote in the first week, with perhaps partial dominance of *b* (parameters −0.94, +1.77). Possible reversal of this effect late in incubation is suggested by some of the results (combined crosses in DG2, $P < 0.10$, pure + cross matings of 6D in DIS, $P < 0.01$).

Although the separate matings show general agreement in the existence of an effect of the *II* × *III*

in purebreds, in crossbreds, and in pure + crossbred matings of substrains 6D and 6F, and the probability of the variance set of parameters

DIS (days 20—21)					TEM (days 1—21)				
ALL	6D + 6F	5D × 6D + 5F × 6F	6D + 5D × 6D	6F + 5F × 6F	ALL	6D + 6F	5D × 6D + 5F × 6F	6D + 5D × 6D	6F + 5F × 6F
−0.6	−0.7	−0.4	−0.7	−0.6	+0.0	+0.1	+0.4	+0.2	−0.7
+0.6	+0.8	+0.1	+1.1	+0.6	+0.4	+1.9	−1.2	+1.1	+0.6
<.10	—	—	—	—	—	—	—	—	—
−0.2	−0.6	−0.1	+1.5	−0.2	+0.1	−0.7	+0.4	+1.5	−0.8
+0.3	+0.6	+0.2	+0.3	+0.8	+0.6	+2.2	−0.6	+0.3	−0.0
—	—	—	<.01	—	—	—	—	—	—
+0.5	+1.0	+0.0	+1.1	+0.6	+0.8	+2.6	−0.7	+1.6	+0.3
−0.5	−0.9	−0.2	−0.7	−0.3	−1.2	−2.0	−0.5	−1.5	−0.6
—	—	—	—	—	—	∼.10	—	—	—
−0.0	−0.0	+0.2	−1.8	+0.2	+0.6	−0.2	+1.4	+0.6	+0.0
−0.1	+0.1	−0.3	−0.1	−1.1	−0.6	+0.4	−0.9	−1.0	+0.5
−0.0	+0.1	−0.5	+3.3	−0.3	−0.1	−1.1	−0.1	+0.9	+0.3
−0.1	−0.6	+0.4	−1.0	+1.4	+0.1	+0.7	−0.3	+0.1	−1.6
—	—	—	<.01	—	—	—	—	—	—
−0.0	+0.4	+0.0	−0.1	−0.8	−0.1	+2.1	−0.6	−0.3	−0.5
+0.2	−0.4	+0.5	−0.2	+0.9	−0.0	−2.1	+1.1	+0.0	−0.2
−0.0	−0.6	−0.2	+1.3	+0.6	+0.7	−1.1	+0.8	+1.9	+0.7
−0.4	+0.1	−0.5	−0.8	−0.8	−0.7	+1.2	−1.6	−1.1	−0.6
—	—	—	—	—	—	—	—	—	—
−0.1	+1.1	−0.9	+3.6	−1.8	+1.1	+2.5	−0.5	+2.8	+0.5
−0.7	−2.1	+0.2	−2.4	+0.5	−1.8	−2.3	−1.2	−3.2	−0.9
−0.7	−1.5	+0.1	−1.5	+0.5	−1.0	−0.2	−0.5	−1.8	+1.0
+0.8	+1.7	+0.1	+1.0	+0.2	+1.6	+1.1	+1.4	+2.5	−1.3
<.10	<.10	—	<.01	<.10	—	—	—	—	—

Table 4. *Estimated relative embryonic mortality parameters (in angles) of dam egg-white triple homozygotes,*

			DG2 (days 16–20)				DIS (days 20–21)				TEM (days 1–21)			
Tf	*II*	*III*	6D	6F	5D × 6D	5F × 6F	6D	6F	5D × 6D	5F × 6F	6D	6F	5D × 6D	5F × 6F
b	*b*	*b*	+0.8	+1.1	+0.4	+2.5	−5.6	+0.6	−0.0	+1.8	−3.6	+0.4	+0.6	+2.8
b	*b*	*a*	+1.8	−1.6	−0.8	+0.6	+5.3	+1.1	−0.0	−1.3	+5.1	+1.0	+1.1	+0.5
b	*a*	*b*	+1.7	−1.4	−0.4	−2.4	+3.2	+0.8	−0.3	−1.3	+3.8	−0.6	−1.2	−2.7
b	*a*	*a*	−5.5	+1.1	+1.1	+0.9	−3.8	−1.1	+0.1	+0.6	−7.2	−1.4	+1.4	+0.6
a	*b*	*b*	+0.2	+1.2	—	−0.5	+7.6	−2.3	—	−2.0	+7.3	+0.5	—	−1.5
a	*b*	*a*	−0.9	—	+0.5	−2.7	−6.8	—	+0.3	+1.3	−5.9	—	+0.6	−1.7
a	*a*	*b*	−1.9	—	—	+0.6	−4.2	—	—	+1.0	−5.0	—	—	+2.1
a	*a*	*a*	+4.7	—	−0.6	+0.4	+4.1	—	−0.2	−0.2	+5.9	—	−0.5	−2.3
		P	.28	.78	.91	.08	.02	.18	.83	.90	.21	.84	.89	.48

interaction late in incubation (Table 2), the probability level in all matings combined (Table 3) is only $P < 0.10$ in both DG2 and DIS. The effect is most marked in the 6D dams (pure + cross, Table 3), where the effect ($P < 0.01$) in the latest period (DIS) receives confirmation at $P < 0.05$ in the preceding DG2 period. The *b*, *a* and *a*, *b* homozygotes are superior, while *b*, *b* and *a*, *a* are inferior. In both periods the four parameters sum almost to zero, indicating that the genetic interaction is additive × additive. Consideration of the results in Tables 2 and 3 suggests that there is reasonable agreement between matings in this effect, with only 5F × 6F differing enough to lead to the non-significance in all matings combined.

None of the other three components of the factorial analyses (locus *III*, interactions *Tf* × *II*, *Tf* × *III*) reaches even near-significance in all matings combined, and for this reason we doubt the validity of the majority of the significances for them recorded in the smaller groupings (Tables 2, 3). Most of the apparent effects appear to be chance fluctuations which show no consistency. One effect, of *III* in days 1–7 and 16–20 and in the Total Embryonic Mortality (days 1–21), is consistent over the pure matings, however, since it occurs in their combination (6D + 6F, Table 3). It may therefore possibly be real, although it certainly does not occur in the crosses. As with *Tf*, the significant effect in the first week is apparently additive (parameters −1.30, +1.49).

The results for the three-way interaction parts of the analyses are given in Table 4. The estimates of the relative embryonic mortality parameters for the dam triple homozygotes are shown for all the single and combined populations, but in only the periods DG2, DIS and TEM (days 16–20, 20–21 and 1–21). The earlier periods FWD and EDG are omitted since no population or combination shows a three-way interaction even approaching significance in these periods. Exact values of probabilities, calculated to the nearest 1% by interpolation, are given as a guide to the confidence which may be placed in each set of parameters.

In each analysis, the parti nvolving the three-way interaction is particularly dependent on the other parts. This follows from the fact that embryonic relative mortality parameters for dam triple homozygotes are fitted by estimating them relative to single heterozygote double homozygotes, relative to double heterozygote single homozygotes, relative to the triple heterozygote. The significance of the variance associated with a set of three-way interaction parameters will thus depend not only on their size and the frequencies of the triple homozygotes, but also on how good the estimates are of the mortalities of all those other triple genotypes relative to which they are estimated. This interdependence has two important consequences, both of which give especial value to the analyses of combined populations. First, significance for the three-way interaction in a combined population will be likely to occur only if there is reasonable agreement between the constituent populations in both these sets of estimates. The second consequence concerns absence of some genotypes, which happened in two of the four single populations (Table 4). Three parameters in 6F and one in 5D × 6D could not be estimated because the corresponding dam triple homozygotes were absent. In addition, another parameter (*a*, *a*, *b*) in 5D × 6D could not be estimated, although this homozygote was present, because of the absence of one of the three single heterozygote double homozygotes with which it was compared (*a*, *ab*, *b*). Such absences not only directly reduce the effectiveness of estimation of the three-way interaction variance, but also lead to distortion of the estimates of some two-way interaction parameters, since these are then based on a markedly incomplete range of double homozygotes. This distortion in turn produces distortion of the three-way estimates. Thus the increase in accuracy of estimation when populations are combined is greater for the three-way interaction than for the simpler effects, since there is not only more data to estimate all mortalities, but also a complete set of genotypes in every case. The latter fact allows a better fitting of the two-way parameters, with a consequent improvement in estimation of the three-way parameters.

and the probability of the three-way interaction variance associated with each set of parameters

DG 2 (days 16–20)					DIS (days 20–21)					TEM (days 1–21)				
ALL	6D + 6F	5D × 6D + 5F × 6F	6D + 5D × 6D	6F + 5F × 6F	ALL	6D + 6F	5D × 6D + 5F × 6F	6D + 5D × 6D	6F + 5F × 6F	ALL	6D + 6F	5D × 6D + 5F × 6F	6D + 5D × 6D	6F + 5F × 6F
+0.9	+0.2	+2.3	+0.8	+0.9	−0.5	−1.9	+0.5	−4.5	+1.7	−0.1	−2.0	+1.9	−2.7	+1.4
+0.4	+0.8	−0.5	+0.6	+0.5	+1.0	+2.9	−0.2	+3.4	−0.8	+1.8	+3.7	−0.1	+2.7	+1.0
+0.2	+1.0	−0.8	+1.3	−1.7	+1.0	+2.7	−0.2	+1.9	−0.3	+0.5	+2.5	−1.0	+1.8	−1.8
−1.3	−3.0	+0.2	−2.4	+1.7	−1.3	−3.1	+0.0	−1.8	−0.6	−2.3	−5.2	−0.2	−2.9	+0.1
+0.9	+2.3	−1.8	−0.3	+2.2	+0.7	+1.8	−0.3	+7.2	−2.5	+2.1	+4.4	−1.0	+6.2	+0.1
−1.7	−2.3	−0.0	−0.5	−2.9	−1.3	−3.0	−0.1	−5.1	+1.4	−2.7	−4.2	−0.6	−4.4	−1.6
−2.1	−3.0	−0.2	−2.2	−0.6	−1.9	−3.3	−0.6	−3.5	−0.1	−2.5	−3.8	−0.3	−4.6	+0.9
+2.9	+4.7	+0.6	+2.8	−0.6	+1.8	+3.4	+0.5	+2.5	+1.0	+3.0	+4.7	+1.0	+3.8	−0.6
.06	.26	.16	.39	.19	.04	.05	.77	<.001	.29	.03	.11	.63	.22	.52

With these considerations in mind, we can now examine the three-way results in detail. The only significant effect in the single populations is in 6D, over days 20–21 (P 0.02). Four triple homozygotes are markedly superior to the group of single, double and triple heterozygotes, and four are markedly inferior. A similar pattern of parameters is seen in 6D over the preceding DG2 period (P 0.28), with the exception of one triple homozygote (*b, b, b*); and the overall mortality (TEM) shows the same pattern as in DIS, at P 0.21. Two other trends or near-significances can be seen. In 6F in DIS, at P 0.18, there is agreement with 6D in the signs of 3 out of the 5 parameters. In 5F × 6F, at P 0.08, there is less agreement with 6D in the same period; the signs of only 4 out of 8 parameters agree, and even among these the larger values in 5F × 6F are the smaller in 6D, and *vice versa*.

Despite these disagreements between the single populations in the partial periods DG2 and DIS, all matings combined (ALL) shows a significant effect (P 0.04) in DIS and a near-significant effect (P 0.06) in DG2. In each case the pattern of parameters is the same as in 6D alone, and as in that population the two periods differ only in the sign of the estimate for the *b, b, b* homozygote. In DIS the combined pures (6D + 6F) show the same pattern as ALL, although at slightly less significance (P 0.05), suggesting that the partial agreement between the parameters for 6D and 6F underestimated the real agreement between these populations, because of the absence of some genotypes from 6F. The increased significance in ALL over 6D + 6F indicates further agreement elsewhere in the data, and indeed the same pattern of parameters is seen in 6D + (5D × 6D) at $P < 0.001$. This high significance seems surprising in view of the fact that 5D × 6D alone showed no evidence of a three-way interaction. However, it should be recalled that two genotypes were missing from 5D × 6D; and since one of these was a single heterozygote double homozygote, the data for a non-missing triple homozygote could not be used. It is clear that these shortcomings in the 5D × 6D analysis led to considerable distortion of the three-way parameter estimates, a distortion the more extreme because there clearly is three-way interaction in 5D × 6D, as evidenced by the way in which the data combine in 6D + (5D × 6D). The high significance in this combination of pure + cross matings of 6D dams indicates a much closer agreement between them than was apparent in the separate analyses. As explained above, this agreement must extend to other genotypes besides the triple homozygotes, a conclusion which receives confirmation from the cluster of significances for 6D + (5D × 6D) over days 20–21 in Table 3.

Returning to DIS in Table 4, the decreased significance in ALL as compared with 6D + (5D × 6D), and the agreement between 6D and 6F, indicate that 5F × 6F disagrees markedly with the other three single populations. This conclusion is confirmed in the double combinations including 5F × 6F, both of which show low significance and a quite different pattern of parameters from 6D or the other combinations. Indeed, the pattern in 5F × 6F alone, which although quite non-significant is a complete set, is the exact converse of that in 6D. The same agreements and disagreements are seen in DG2, although less strikingly than in DIS. All matings combined, 6D, and the double combinations excluding 5F × 6F all show closely similar patterns of parameters, while 5F × 6F and the double combinations including it show patterns differing considerably from the foregoing.

In view of the general agreement among 3 out of 4 populations over the two late periods DG2 and DIS, and the absence of even near-significances in the earlier periods FWD and EDG, it is not surprising that the same pattern of parameters is seen over the whole incubation period (TEM), with just the same agreements and disagreements between populations and combinations. The significant (P 0.03) three-way interaction effect in ALL in TEM is thus clearly also derived from agreement between 3 out of 4 populations; and it represents our main finding on the final selective effect of the three egg-white loci through differential embryonic mortality. The estimated parameters sum to −0.2 in all matings combined, and to +0.1 in the pures alone (6D + 6F, P 0.11), so that

the selective effect demonstrated is of a genetically additive × additive × additive interaction.

Discussion

Associations of dams' egg-white genotypes with embryonic mortality have been found in various parts or the whole of the 21-day incubation period. They include main effects of the individual loci as well as some genetic interactions between them. In their purebred matings the two substrains agree markedly in the nature and direction of all the effects found. However, this agreement extends to both sets of crossbred matings in only one instance, namely, the additive effect of *Tf* over days 1—7; and here they show the effect to a greater degree than the purebred matings. Otherwise, the tendency is for the agreement between the purebreds to extend to one or other set of crossbred matings, but not both. Thus the main effect of *II* over days 1—7 occurs in both pures and in 5F × 6F, but not in 5D × 6D. On the other hand, both the *II* × *III* and the *Tf* × *II* × *III* interactions show remarkable agreement in the signs of the parameters over days 16—21 (DG2 + DIS) in both pures and in 5D × 6D, but not in 5F × 6F. In the case of the three-way interaction the same agreement occurs in the Total Embryonic Mortality. The only effect to occur in both pures but neither cross is the least certain of those considered, namely, the possible additive effect of *III* over days 1—7. There is thus little evidence for consistent differentiation between purebred and crossbred matings.

The present results may be compared with the findings in our study of the pure matings of the ancestor strain 6 four generations previously (Morton *et al.*, 1965). Unfortunately, the main effects at that time were demonstrated over days 6—15, a period which could not be separately analysed in the present data because mortality was now so low. Nevertheless, some useful comparisons can be made. In strain 6, the *b* homozygote for *Tf* was significantly superior to the *a* homozygote over days 6—15. This is not inconsistent with our present finding that the *a* homozygote is superior to the *b* early in incubation, since this effect occurs significantly in only the very earliest period (days 1—7), while in later periods there is now some indication of reversal in the purebreds, thus agreeing with the result in 6. No significant main effects of *II* and *III* were seen in 6, but the signs of the parameters were in general agreement with those now found in the pures. This statement also applies to the *II* × *III* interaction effect. On the other hand, the highly significant ($P < 0.01$) *Tf* × *III* interaction effect seen in strain 6 over days 6—15 has no parallel in the present results. The only other significant effect in 6 was the three-way interaction over days 6—15, the period for which there is no present counterpart, and the signs of the parameters found then show little agreement with the main pattern seen in the present data over days 16—21 and 1—21. A close agreement is seen, however, when the data for the corresponding periods in strain 6, especially 16—20 and 1—21, are compared with the present results.

The effects now reported are therefore consistent not only over the pure matings of the two substrains, but also over the pure matings of the ancestor strain 6. The absence of significance for these effects in 6 may have resulted from the small size of the data (175 dams, 3632 fertile eggs), and is paralleled in the present work when the substrains are considered separately. As pointed out above, most of the effects are also consistent over one or other or both sets of crossbred matings. This general consistency of egg-white effects contrasts with the changes in the blood-group effects between the two generations and the two substrains (Gilmour and Morton, 1970). As discussed in the paper cited, the polyallelic *B* locus seems to be readily responsive to quite small changes in the environmental conditions and the rest of the genome; while it now appears that the egg-white loci are more stable in their selective effects, perhaps because they appear to form an integrated interacting system.

In examining possible mechanisms for the maintenance of stable polymorphism of the three loci, the important selective effects to consider are those operating on the total embryonic mortality (TEM). The significant effects of the single loci *Tf* and *II* were restricted to the first high mortality period (the first week), while in the latter part of incubation when mortality was again high there were indications of reversal of these effects. Consequently no effects were demonstrable on TEM. Multiplicative combination of opposite effects must lead to some degree of dominance, which might in some circumstances go as far as overdominance. If this is so for *Tf* or *II*, any such overdominance is too small for detection in our data. In contrast, the significant additive effect of *III* in the first week was not reversed later in incubation, so that a near-significant ($P \sim 0.10$) additive effect in the same direction (*a* homozygote superior, *b* inferior) was seen in TEM (days 1—21). If this effect is a real one, maintenance of polymorphic equilibrium at this locus must depend on other balancing effects, including, presumably, the interactions.

The two proven interaction effects (*II* × *III* and the three-way) occurred late in incubation and probably acted over most of the last six days when there was high mortality, but only the latter was demonstrable in TEM. Thus the values of the parameters for the three-way interaction for TEM in all matings combined (Table 4) are our only estimates of the selective effects of the egg-white loci. They represent the estimated mortality differences of the eight triple homozygotes after allowance has been made for effects of the loci singly and in two-way interactions. Since none of the latter effects is significant in TEM, there is no evidence for any overall mortality differences between any of the genotypes with at least one

locus heterozygous, i. e., the triple heterozygote and the single and double homozygotes; and the parameters for the three-way additive interaction represent comparisons of the eight triple homozygotes with the average mortality ascribable to any of the other 19 genotypes.

Polymorphism could be maintained by some overdominance at each locus, which might be too small to be detected individually or when the loci are considered two at a time, but possibly detectable when three are considered multiplicatively. In the latter case there should be superiority of the triple heterozygote and general inferiority of triple homozygotes. Neither of these is seen in our data, as shown above and because the three-way parameters sum to −0.2, essentially zero. If simple multiplicative overdominance is a factor in the maintenance of the polymorphisms, we have not succeeded in demonstrating it. Lack of overall superiority of the triple heterozygote might result from the situation envisaged by Sved, Reed and Bodmer (1967) and King (1967). In separately proposed models for balanced polymorphism of numerous loci they assumed that the overdominance per locus was small (selection differential $\sim 1\%$), and that epistasis operated such as to restrict but not extinguish the superiority of the multiple heterozygote. The corollary of this situation, stressed particularly by King, is that the overdominance per locus necessary to maintain polymorphism is only expressed in genotypes with many loci homozygous, while the other genotypes occur in a very short range of the fitness distribution. This view is similar to our proposals (Morton *et al.*, 1965), for polymorphism of numerous diallelic loci, namely, that overdominance per locus is small, and that consequent random fixation "may be avoided by interaction between loci, for example such that only the multiple homozygotes are appreciably deleterious". One of our results fits these expectations, i. e., that there are no overall embryonic mortality differences between any of the genotypes with at least one locus heterozygous. On the other hand, only 4/8 triple homozygotes show inferiority, and no single locus shows overdominance when both the other loci are homozygous (TEM in all matings, Table 4). As one explanation of the occurrence of some superior triple homozygotes, we could assume that there was another interacting locus involved. Then for the pairs of quadruple homozygotes including any of the three present superior triplets to be inferior as required by theory, it would be necessary for the corresponding triple homozygote/single heterozygotes to be more superior than the original triplets. Although there is nothing against some genotypes having superiority of this order, it is clear that the general restriction on heterozygote superiority in the hypothesis sets a limit to the number of further loci which could be involved, since each additional locus would require approximate doubling of the multiple homozygote inferiority. An alternative explanation is suggested by the occurrence of both positive and negative deviations (Table 4), namely that the triple homozygotes may be subject, in some generations, to change in their selective value. If extreme values (+ or −) occur in the triple homozygotes only, then multiplicative combination of fitnesses over generations will tend in the long term to raise the geometric mean relative fitnesses of the other genotypes, leading first to some dominance and eventually to inferiority of all multiple homozygotes. This explanation is an extension of the proposal of Haldane and Jayakar (1963) discussed in the preceding paper.

Our results provide evidence that extreme selective values are associated with multiple homozygosity for several polymorphic diallelic loci concerned with similar functions, and hence that such loci interact in their effects on fitness. In some circumstances, if the similarity of their functions is close enough and if multiple alternatives confer particular advantage, such loci might evolve into closely linked complexes (Sheppard, 1953, Gilmour, 1960, Bodmer and Parsons, 1962). This evolution may be occurring among the egg-white loci, thus Buvanendran (1967) has shown that *II* (Lush, 1961) and *Ov* (Lush, 1964b) are rather closely linked (recombination frequency $< 1\%$). *Ov* is not segregating in our populations, so that our data on *II* concern only two out of the four or more possible linkage combinations or pseudoalleles. Tightening of linkage is one rather specialised consequence of interaction between loci and may be related to special needs of the population for rapid adaptability and lead to polymorphisms of multiple alleles. On the other hand, we believe that many loci interact and do not become linked. These include the three types of numerous segregating loci that are recognised, the one directly identifiable, controlling variants of antigens, proteins and enzymes; the other two inferred, and consisting of the polygenic loci responsible for quantitative variation, and the systems of modifying genes invoked whenever the activity of a major gene can be altered by selection. There seems no reason to make any functional distinction between any of these: the polymorphisms appear different merely because they can be specifically identified. We are not alone in this approach, which has been used by several other authors recently. We do however wish to emphasize the important role played by epistasis in the maintenance of segregation and hence of genetic variability.

In the preceding paper (Gilmour and Morton, 1970) we concluded that associations of genotypes rather than genes or serological factors should have been studied in the work on polyallelic polymorphisms in animals and man which we cited (Neimann-Sørensen and Robertson, 1961; Stansfield, Bradford, Stormont and Blackwell, 1964; Morton, Krieger and Mi, 1966). We have now made the case that the failure of these authors to obtain evidence of selective effects at

single diallelic loci is to be expected. Analytical designs which examined interactions between loci might have yielded results, particularly in domestic animals where there is always some degree of inbreeding in breeds or strains. The increased general homozygosity of the dams in our closed populations probably contributed to our success in demonstrating interaction effects on fitness. It is less likely that these will easily be found in man, unless perhaps in small partly inbred groups; in this respect the triracial population studied by N. E. Morton *et al.* (1966) was particularly unsuitable.

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Bibliography

1. Bodmer, W. F., Parsons, P. A.: Linkage and recombination in evolution. Adv. Genetics **11**, 1—100 (1962). — 2. Buvanendran, V.: Linkage relationships of egg albumen loci in the domestic fowl. Brit. Poultry Sci. **8**, 9—13 (1967). — 3. Gilmour, D. G.: Blood groups in chickens. Brit. Poultry Sci. **1**, 75—100 (1960). — 4. Gilmour, D. G., Morton, J. R.: 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. Genet. Res. **15**, 265—284 (1970). — 5. Haldane, J. B. S., Jayakar, S. D.: Polymorphism due to selection of varying direction. J. Genet. **58**, 237—242 (1963). — 6. Harvey, W. R.: Least squares analysis of data with unequal subclass numbers. U. S. Dept. Agr., Agr. Research Serv. Publ. No. ARS-20-8 (1960). — 7. King, J. L.: Continuously distributed factors affecting fitness. Genetics **55**, 483—492 (1967). — 8. Lush, I. E.: Genetic polymorphisms in the egg albumen proteins of the domestic fowl. Nature **189**, 981—984 (1961). — 9. Lush, I. E.: Egg albumen polymorphisms in the fowl: loci *II* and *III*. Genet. Res. **5**, 39—49 (1964a). — 10. Lush, I. E.: Egg albumen polymorphisms in the fowl: the ovalbumin locus. Genet. Res. **5**, 257—268 (1964b). — 11. Morton, J. R., Gilmour, D. G., McDermid, E. M., Ogden, A. L.: Association of blood-group and protein polymorphisms with embryonic mortality in the chicken. Genetics **51**, 97—107 (1965). — 12. Morton, N. E., Krieger, H., Mi, M. P.: Natural selection on polymorphisms in Northeastern Brazil. Amer. J. Hum. Genetics **18**, 153 to 171 (1966). — 13. Neimann-Sørensen, A., Robertson, A.: The association between blood groups and several production characteristics in three Danish cattle breeds. Acta Agr. Scand. **11**, 163—196 (1961). — 14. Ogden, A. L., Gilmour, D. G., Morton, J. R., McDermid, E. M.: Inherited variants in the transferrins and conalbumins of the chicken. Nature **195**, 1026—1028 (1962). — 15. Sheppard, P. M.: Polymorphism, linkage and the blood groups. Amer. Naturalist **87**, 283—294 (1953). — 16. Stansfield, W. D., Bradford, G. E., Stormont, C., Blackwell, R. L.: Blood groups and their associations with production and reproduction in sheep. Genetics **50**, 1357—1367 (1964). — 17. Sved, J. A., Reed, T. E., Bodmer, W. F.: The number of balanced polymorphisms that can be maintained in a natural population. Genetics **55**, 469—481 (1967). — 18. Williams, J.: A comparison of conalbumin and transferrin in the domestic fowl. Biochem. J. **83**, 355—364 (1962).

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