INFLUENCE OF B LOCUS BLOOD GROUPS ON ADULT MORTALITY AND EGG PRODUCTION IN THE WHITE LEGHORN CHICKEN

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ABSTRACT

The influence of the B locus blood group on adult viability and egg production was studied in two White Leghorn populations (S1 and S2) synthesized from inbred line crosses. Each line segregated for four B alleles. Four homozygotes and six heterozygotes were produced in each line over a five-year period, and for an additional three years tests on certain blood-group combinations were continued. A total of 4371 birds were included in the study. Greatest differences in blood groups were found in the S1 line, with the Bz and Bz1 alleles seemingly having favorable effects and with B' having unfavorable effects. The B' homozygote was consistently the lowest in egg production (53.2%) and highest adult mortality (40.4%). The relative spread in standard deviation units between the B' and Bz homozygotes was more than three times greater in adult mortality than in egg production; Bz was incompletely dominant to B'. Within the S1 line, the superiority of the heterozygotes was mainly a consequence of the poor fitness of the B1 homozygote, suggesting that in a random-mated population B1 would be maintained only by mutation and not by a polymorphic mechanism.—Over the eight years of the experiment, adult viability of the B1 homozygote improved 4.4% per year (P < 0.05). Assuming this regression results from natural selection, either of two hypotheses can account for the results: (1) The B locus is pleiotropic with natural selection for many B modifiers, and (2) the B locus is neutral but linked to a major fitness locus.

CHICKENS have at least twelve blood-group systems each with two or more alleles per locus (GILMOUR 1970; CRITTENDEN, BRILES and STONE 1970). Evidence indicates that the multi-allelic B system, more than any other, has fitness value (SCHULTZ and BRILES 1953; BRILES, ALLEN and MILLEN 1957; ALLEN and GILMOUR 1962; GILMOUR 1959; BRILES and ALLEN 1961; OKADA and MATSUMOTO 1962). Also, B functions as a histocompatible system (SCHIERMAN and NORDSKOG 1961, 1963, 1964), corresponding to the H-2 system in mice.

One of the most striking effects at the B locus, reported by BRILES and ALLEN (1961), was the very high mortality of the homozygous B' individuals after they reach laying age but not before. Other B alleles also seemed to show either superiority or inferiority in homozygous combinations as juveniles or as adults.
They suggested that the differential effects of B alleles at different ages may be an important mechanism by which polymorphisms are maintained. Measured over the entire life-span, the heterozygote would be superior to the homozygote. Their study covered data collected over a three-year period, but this included only 25 $B'$ homozygous females. Thus, year effects and small numbers might have biased their conclusions.

Gilmour and Morton (1970) studied the effects of the B blood-group system on embryonic mortality in two breeds of chickens. No effect of genotypes of dams on viability could be detected, but differences between genotypes of embryos seemed to have an important influence on embryonic mortality. Also, certain genotypes which were initially superior became inferior in later generations, and vice versa. They hypothesized that both epistasis and genotype × environment interactions are involved in the mechanism of a stable B polymorphism.

All this points to a complex adaptive mechanism associated with the B blood-group locus. Two alternative interpretations would be (1) that natural selection for B modifiers changes the pleiotropic effect, and (2) that the B genes are neutral but linked to major fitness gene.

The literature on fitness in chickens as related to blood groups is mostly based on inbred populations; yet, these may not be the most desirable experimental material for such studies even though each inbred line, in itself, might be near a genetic equilibrium. That is, one line could be in a coupling phase and another could be in repulsion phase with respect to a fitness gene and a blood group gene.

Randombred or outbred populations, being nearer to a genetic equilibrium, at least theoretically, might be considered better experimental material than inbreds. However, true “equilibrium” populations are probably nonexistent. Furthermore, an outbred population presents certain technical difficulties in developing single-specificity, blood-typing reagents.

An additional possibility would be to synthesize a population, segregating for known blood-group genes, from crosses of well-typed inbred lines. This would eliminate the uncertainty as to number and frequency of B alleles in an outbred population. Compared to inbreds, synthetics would be more homeostatic and the possibility of interactions of blood groups with the specific genetic background of an inbred line would be lessened. Moreover, synthetic populations—being easier to reproduce than inbreds—would simplify the problem of producing adequate numbers for meaningful experimental comparisons.

In forming a synthetic line from the cross of two inbred lines, one must consider the possible consequences of linkage disequilibrium if, in fact, linkages of blood group loci with fitness loci exist. Tight linkages might not be distinguished from pleiotropy. If there is less than tight linkage, then a synthetic population, over succeeding generations, should change in fitness as linkages break up. Alternatively, if there is natural selection for genes which modify the pleiotropic effect of the B locus, a synthetic population should change in fitness over generations.

The objective of the experiment we report here is to study fitness of several B blood group genotypes in two synthetic populations. Because the number of
segregating B alleles is never certain in an outbred population, the two special populations developed for this study each segregated for four known B alleles. Of special interest was the low-viability, $B'$ homozygous genotype already alluded to (Briles and Allen 1961), and the plan was to see whether its fitness might gradually improve over generations. If so, this would be evidence for either linkage or selection of modifier genes.

**MATERIALS AND METHODS**

*Populations:* The two populations, S1 and S2, were derived from crosses of three inbred lines obtained from a commercial source:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Line</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B'B'B'$</td>
<td>S1</td>
<td>($B', B_2, B_3, B_4$)</td>
</tr>
<tr>
<td>$B'B'B'$</td>
<td>S2</td>
<td>($B_4, B_5, B_6, B_7$)</td>
</tr>
</tbody>
</table>

Note that S1 and S2 have two alleles in common ($B_5$ and $B_7$) derived from the sire lines, but each has two different alleles derived from their respective dam lines.

Eggs from the single-cross foundation matings were produced in 1963 and hatched at the University Poultry Farm. The chicks were blood-typed at three to four months of age with reagents initially supplied by the commercial breeder. In 1964, specific antisera were produced in quantity from isomunizations for each of the alleles in both S1 and S2 populations. Antisera with single specificities were obtained by appropriate absorptions.

*Mating scheme:* In 1964, female heterozygotes of each line mated to nonsib male heterozygotes produced all ten kinds of possible genotypes: four homozygotes and six heterozygotes in each line. Primary interest was focused on the difference in performance between the B blood-group genotypes.

Within each line, breeders in laying condition were selected solely on their B genotypes. The breeding plan, designed to avoid the formation of sublines and to minimize inbreeding, avoided full-sib and half-sib matings.

Each year, heterozygous sires were mated to equal numbers of homozygous and heterozygous dams. Matings were arranged in four sets with each set restricted to three of the four alleles segregating in the populations. For example, in the S1 population, the first mating set consisted of birds carrying alleles $B_5, B_7$, and $B_{15}$, but lacking the $B_{16}$ allele; the second mating set lacked $B_{15}$; the third lacked $B_5$; and the fourth lacked $B'$. Similarly, in the S2 population, one of the four alleles—$B_{16}, B_{19}, B_{21}$ and $B_5$—was deleted in each of four sets of matings. Restricting the matings in this way better equalized the number of progeny produced per genotype, compared with the expected distribution of progeny per genotype from an unrestricted or complete diallel mating between all ten genotypes.

In 1966 the mating scheme was modified slightly because the birds were kept in single cages and were artificially inseminated. The number of sires were changed to 24 from 12 in each line to increase the supply of semen. Thus, each sire inseminated 6 females rather than 12 as in 1965.

Because the mortality of the $B'$ homozygote was much higher than of the others, additional matings were made to obtain larger numbers of them in the last five years of the study. In the supplementary years of 1970, 1971 and 1972, only $B'$ homozygotes and heterozygotes were produced. The number of genotypes per line per year averaged about 40 for a total of 3814 pullets of all genotypes in the first five years and a grand total of 4371 pullets observed over the eight years of the study.

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* Hy-Line Poultry Farms, Johnston, Iowa.
* We follow the convention adopted by most immunogeneticists of using superscripts to denote blood-group alleles.
Standard methods were used for blood-typing. In 1963 and 1964, 10 x 77 mm tubes were used, and starting in 1965, plastic plates containing 2-ml wells in 10 rows of 10 were used.

**Flock management and records:** The chicks were produced in two or three hatches in each generation, reared at the University Poultry Farm, and blood-typed during the growing period. In 1965 and 1966, pullets were housed on the floor and trapnested. In the succeeding years test pullets were kept in cages.

Egg production records (2 to 4 days per week) were obtained from housing to about 475 days of age. Hen-day egg production was calculated as the percentage of eggs laid to the total number of days.

**RESULTS**

The mean egg production and adult mortality of the ten genotypes of each line, averaged over the first five years of the results, are plotted in Figure 1. The range among genotypes for mortality was 3.5 standard deviations,1 but only a little more than one standard deviation for egg production. The correlation between egg production and the mortality level of the genotypic means was 0.80; this rather strong correlation is mainly a consequence of the extreme values of the outlying genotypes \( B^1B^4 \), \( B^2B^2 \) and \( B^{*1}B^{*1} \) of the S1 line as shown in Figure 1. Otherwise, the genotypic averages are closely clustered around the population mean. For S2, the range of genotype values was less than 1.0 standard deviation for survival and 0.5 for egg production.

![Figure 1](image-url)  
_Figure 1._ Mean deviation in egg production and adult mortality of \( B \) locus blood group genotypes. The more extreme genotypes are identified. Fitness of the \( B^{*1}B^{*1} \) genotype seems to be influenced by the genetic background of the line in which it occurs. Mean egg production is 58.0% and mean mortality is 16.3%.

1 Standard deviations for egg production and laying-house mortality are based on the intra-line and year mean differences between genotypes.
The most striking result was the relatively high mortality and low egg production of the $B'$ homozygote in the S1 line, confirming the findings reported by Briles and Allen (1961). The $B'$ allele was nearly recessive as regards adult mortality and egg production. The $B'$ homozygotes had 54% mortality, compared with 10.6% for the $B^i$ heterozygotes and 6.8% for the non-$B'$ genotypes. For egg production, the $B'$ homozygotes averaged 40.4%, compared with 53.4% for the $B^i$ heterozygotes and 56.0% for the non-$B'$ genotypes. In the S2 line, the differences between homozygotes were significant ($P < .05$) for egg production but not for mortality. Differences between heterozygous genotypes were significant in S1 but not S2. In S1, superiority of the heterozygotes mainly reflected the exceptionally poor performance of the $B'B'$ genotype.

From a hierarchical analysis of variance on the first five years of the data in the S1 line, differences between genotypes within full-sib groups were tested statistically. Table 1 demonstrates highly significant mean squares for years, sires, dams and genotypes within full-sib groups. Blood-group genotypes accounted for 12.9% of the variance in mortality and 9.7% of the variance in egg production. The error term, "full-sibs within dams," was used to test the mean squares for dams and, "within genotypes and full-sib groups," was used to test genotypes within full-sib groups.

In Figure 2 the regression of the difference in egg production and adult mortality between the $B'$ homozygotes and heterozygotes is shown over eight generations. The decrease in adult mortality of 4.4% per generation is statistically significant ($P < .05$). Thus, it seems that the $B'$ allele has been gradually improving in fitness through selection of modifier genes or because B is linked to a major fitness locus.

### Table 1

**Analysis of variance of adult mortality and egg production in the S1 line on five years of data, 1956–1969**

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Mean squares</th>
<th>Variance components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Actual Percent</td>
</tr>
<tr>
<td>Adult mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years</td>
<td>4</td>
<td>1.2656**</td>
<td>0.0027</td>
</tr>
<tr>
<td>Sires/Y</td>
<td>73</td>
<td>0.2312**</td>
<td>0.0037</td>
</tr>
<tr>
<td>Dams/S</td>
<td>471</td>
<td>0.1395**</td>
<td>0.0103</td>
</tr>
<tr>
<td>Full sibs/D</td>
<td>1377</td>
<td>0.1033</td>
<td></td>
</tr>
<tr>
<td>Genotypes/FS</td>
<td>539</td>
<td>0.1207**</td>
<td>0.0161</td>
</tr>
<tr>
<td>Within G and FS</td>
<td>838</td>
<td>0.0922</td>
<td>0.0922</td>
</tr>
</tbody>
</table>

| Egg production   |    |              |                     |
| Years            | 4  | 0.97927**    | 0.0024              |
| Sires/Y          | 73 | 0.11731**    | 0.0031              |
| Dams/S           | 459| 0.04503**    | 0.0030              |
| Full Sibs/D      | 1277| 0.03492      |                     |
| Genotypes/FS     | 506| 0.03950**    | 0.0044              |
| Within G and FS  | 771| 0.03193      | 0.0319              |
|                  |    |              | 0.0448              |

100—
The \( B^{19} \) and \( B^{21} \) alleles, being common to both lines, permitted a comparison of
the three genotypes \( B^{19}B^{19}, B^{21}B^{21}, \) and \( B^{19}B^{21} \) in two somewhat different genetic
backgrounds tested over five years. The analysis of variance (Table 2), based on
the assumption that genotypes are fixed effects while years and lines are random
effects, resulted in significant mean squares for Genotypes, Lines and Geno-
types \( \times \) Lines for egg production but not for adult mortality.

**Table 2**

*Analysis of adult mortality and egg production as influenced by the B locus genotypes
\( B^{19}B^{19}, B^{21}B^{21}, \) and \( B^{19}B^{21} \) in two lines over five years*

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>( df )</th>
<th>Mean squares Egg production</th>
<th>Mean squares Adult mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes ( B^{19} )</td>
<td>2</td>
<td>118.3**</td>
<td>252.5</td>
</tr>
<tr>
<td>Lines</td>
<td>1</td>
<td>616.5**</td>
<td>53.4</td>
</tr>
<tr>
<td>Years</td>
<td>4</td>
<td>70.6</td>
<td>121.0</td>
</tr>
<tr>
<td>( G \times L )</td>
<td>2</td>
<td>64.7*</td>
<td>205.6</td>
</tr>
<tr>
<td>( G \times Y )</td>
<td>8</td>
<td>14.5</td>
<td>51.6</td>
</tr>
<tr>
<td>( L \times Y )</td>
<td>22</td>
<td>15.6</td>
<td>15.9</td>
</tr>
<tr>
<td>( G \times L \times Y )</td>
<td>8</td>
<td>5.2</td>
<td>102.5</td>
</tr>
</tbody>
</table>
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to Marek's disease than $B^{i\theta}$ in field tests where a high incidence of disease was present (16% vs. 29%); in other field tests where a low incidence of Marek's was encountered, the difference was less (2.6% vs. 4.5%).

In 1968 and 1969, all birds that died in the laying house were autopsied at the ISU Veterinary Diagnostic Laboratory. The data were not sufficient to draw conclusions as to differences in causes of mortality between the genotypes. In particular, the consistently higher level of mortality of the $B'B'$ genotype could not be clearly related to a higher incidence of leukosis. The specific causes of the higher mortality of this genotype are still unknown.

DISCUSSION

If blood-group genes are pleiotropic or are neutral but linked to separate fitness genes, the observed effects of blood groups would be differently interpreted in terms of genetic mechanisms. If we assume that the B locus is pleiotropic, then some alleles have large and consistent effects on fitness components and others have small effects such that there is a range of fitness values distributed about a zero mean. Presumably most would have small or nearly neutral effects, but rarer alleles might either strongly enhance or reduce fitness. For example, $B'$ and $B^{i\theta}$ reduced fitness but $B^s$, $B^{i\theta}$ and possibly the $B'A$ allele improved fitness. The mean differences between adult survival of the $B'B'$ and $B'B^s$ genotypes in the S1 line was 3.5 standard deviations.

Following the argument of OHTA and KIMURA (1971) each blood group allele may be the result of a unique mutation and not repeated in the same or other populations. This could happen if mutations mainly occur in that portion of the DNA molecule where the possible number of permutations of base combinations is large, and would explain the wide array of B alleles in the fowl and also in the bovine (STORMONT 1958). We are not aware that identical B alleles have ever been discovered in independent populations of chickens. Admittedly, part of the problem is that typing reagents developed for one population of chickens do not usually perform satisfactorily in other populations because of cross reactions.

In general, this study supports the contention of earlier workers that the B blood group has adaptive value. However, simple heterozygote superiority for specific B locus allelic combinations could not be demonstrated. Rather, heterozygote superiority seems to depend on the total genetic composition of the population.

KOJIMA and LEWONTIN (1970) recently considered the case of a neutral gene linked to a fitness gene. If we assume that the B alleles have neutral effects but are linked to a fitness locus with a recombination fraction of about 10% per generation, this model would closely fit the observed data (Figure 2) and could account for the recovery of viability of the $B'B'$ genotype over successive generations. This would be a reasonable hypothesis if $B'$ was initially linked to a mutant gene with low fitness. Such a mutation could have occurred in the founda-

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1 Marek's disease is a part of the avian leukosis complex and is caused by a Herpes virus belonging to the DNA group.
tion inbred line #1. The linkage hypothesis would permit recombinations among all $B'$ heterozygotes, but natural selection could operate only on the homozygotes.

The alternative hypothesis is that $B'$ itself is the mutant gene with low fitness and that natural selection for modifier genes has improved the fitness of $B'$. Again, natural selection could operate only among the homozygotes because $B'$ is a recessive gene. Only about one-eighth of the dams were $B'$ homozygotes, while none of the sires were. Hence, selection would be weak. Furthermore, the accumulation of such modifiers would benefit only the $B'$ homozygotes and not necessarily the other genotypes. In theory, the $B'$ modifiers could be detrimental to other genotypes.

There seems not to be a simple basis for making a choice between the two hypotheses, but for this special synthetic population, where a constant proportion of blood group genotypes was deliberately maintained each generation, the linkage hypothesis seems to provide the simplest explanation for the regression shown in Figure 2.

A number of former and current graduate students contributed to this study. We are indebted to Dr. L. W. Schierman, Dr. C. S. David, Dr. John Marangu, Dr. D. W. Casey, Dr. H. L. French, Mr. C. Y. Lin and Dr. John Tierce. Also we appreciate the services of Dr. David Cox of the ISU Statistical Laboratory for help on the data analyses.

LITERATURE CITED


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