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Disease resistance as related to sex chromosomes and blood groups in the fowl

by

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INTRODUCTION

Individuals may differ genetically in the susceptibility and resistance to various diseases. Some individuals may survive a particular infection while others may die under the same environmental conditions. It has been said that no investigator who has adequately sought genetic differences in disease response has failed to find them (Gowen, 1937).

The genetic mechanisms of disease resistance may be simply inherited or may be polygenic. Both a resistant and a susceptible line to Marek's disease were developed by Cole (1968) in 2 generations of selection. Cellular susceptibility to leukosis/sarcoma viruses is controlled by at least 3, independently segregating, autosomal genes (Crittenden et al., 1972). Fixation of alleles for cellular resistance to the leukosis/sarcoma viruses should help to reduce the incidence of neoplastic diseases.

A seemingly different kind of genetic resistance to disease is associated with the sex chromosome. The female progeny of the Leghorn males, mated to heavy breed females, are consistently higher in laying house mortality than the reciprocal cross. Nordskog and Phillips(1960) presented evidence showing that Leghorn sex chromosome is associated with adult mortality in crossbreds. The Z sex chromosome in the fowl is the fifth largest and represents 10 percent of

the total avian genome. At least 16 separate loci showing Mendlian segregation have been reported (Briggs, 1971). The W chromosome, on the other hand, seems to be devoid of genetic material. There seems not to be any crossing over between the Z and W sex chromosome (Ohno, 1967). In the human, the sex chromosome may carry genes associated with hemophilia and a deficiency in the enzyme, glucose-6-phosphate dehydrogenase, leading to anemia.

Beside producing a blood group substance, the B blood group locus in chickens seems to play a role in the control of certain basic physiological processes. The locus influences both egg production and viability (Rishell, 1968) and is the major locus controlling homograft rejections (Schierman and Nordskog, 1964).

The specific objectives of this study were: (1) to determine the role of the Z sex chromosome and maternal line differences on disease resistance, and (2) to determine whether the B blood group locus is associated with specific diseases and whether it may be a controlling factor in the immune response.

REVIEW OF LITERATURE

Sex Chromosome and Maternal Effects

In general

The possible importance of sex-linkage in quantitative inheritance was emphasized by Beilharz (1962). In chickens, because the sex chromosome holds one sixth of the genome, it should have an important effect on economic traits, such as egg production and body weight. When data on females of the avian species are subjected to an analysis of variance, sex-linkage is postulated if the sire variance component is larger than the dam component.

Jerome et al. (1956) inferred sex-linked effects on sexual maturity in agreement with earlier reports of higher sire than dam components in analysis of variance on age of sexual maturity (Lerner and Taylor, 1943; Lerner, 1945; and Peeler et al., 1955). Hale and Clayton (1965) analyzed a series of diallel crosses and also found a larger sire component for days to first egg. In similar studies egg production was found to be sex-linked (Goodman and Jaap, 1961; Hogsett and Nordskog, 1958; and Jerome et al., 1956).

Body weight of broilers and laying hens was deduced to be sex-linked based on statistical analysis (Goodman and Godfrey, 1956; Hogsett and Nordskog, 1958; Jerome et al., 1956; and Osborne, 1953).

Sex-linkage effects on egg weight were inferred from statistical analysis by Lerner and Cruden (1951), Osborne (1953, 1954), Wyatt (1954), Hicks (1958), Goodman and Jaap (1961), and Hale and Clayton (1965).

Sex-linkage effects can also be deduced from cross-breeding experiments. Differences between reciprocal crossbreds are commonly assumed to result from the Z sex chromosome effect of the male parent. Based on this method Dudley (1944), Kawahara and Ichikawa (1960), and Hutt (1962), have all demonstrated a sex chromosome effect on sexual maturity. The offspring of White Leghorn males and heavy breed females, matured earlier than the progeny of the reciprocal cross.

King (1951) crossed Rhode Island Reds with Barred Rocks reciprocally and noted a difference in egg production. The Barred Rock-sired progeny had higher production suggesting a sex chromosome effect.

Beilharz and McDonald (1961) postulated a sex chromosome effect on body weight in chickens. They reported significant differences between the progeny of reciprocal crosses of the White Leghorn and Australorp.

Body weight in the fowl is also influenced by sex-linked dwarf genes. Experiments with dwarf genes have been reported by Hutt (1959), Godfrey (1953), Custodio and Jaap (1970, 1973), and French and Nordskog (1973).

A recessive dwarf gene (dw) reported by Hutt (1949) reduced body weight by 28 and 43 percent in Leghorn females and males, respectively.

In Rose Comb Black Bantams, Godfrey (1953) reported a sex-linked recessive gene (rg) depressing growth rate before 9 weeks of age and reducing mature body size. Custodio and Jaap (1973) reported a recessive sex-linked dwarfing gene in Golden Sebright Bantams which determines part of the small body size in the bantams. It may be an allele to the original recessive dwarf gene, dw, first reported by Hutt (1949) and may be identical with rg.

There are no conclusive results as to the role of the sex chromosome in egg production. Ghigi (1948) crossed 2 species of Gallus and reported sex-linkage effects on egg weight. Nordskog and Phillips (1960), on the other hand, did not find egg weight differences between reciprocal crosses of Leghorn, heavy and Fayoumi lines.

In all the literature so far considered, sex-linkage effects were inferred from crossbreeding experiments or from analyses of variance of quantitative data, but in either case the conclusion might not be valid. Differences between female progeny of reciprocal crosses have been attributed to sex chromosome effects, but maternal and W sex chromosome effects of the dam line may also be involved. The differences between sire and dam variance components in an analysis of

variance might be due to sex-linkage, dominance or epistatis. Maternal effects can reduce or mask possible sex chromosome effects.

Eisen et al. (1966) worked with analysis of variance models A and B, previously presented by Henderson and Griffing. They showed that using estimates from the 2 models will yield estimates of additive autosomal, sex-linked and maternal effect variances, when data are collected only on heterogametic female progeny as found in avian species. These estimates were considered as crude but not wholly appropriate for species with homogametic females. Briggs and Nordskog (1973) derived special populations from the Fayoumi and a "heavy" breed line. Their design enabled the direct estimation of Z and W sex-chromosome differences between these breeds and the indirect measurement of autosomal and maternal differences between breeds. In addition, separate effects of breed heterosis were estimable. The Z chromosome breed difference was shown to influence age of sexual maturity and rate of egg production and there was some evidence for an effect on early body weight, but sex-linkage seemed not to affect adult body weight.

A maternal effect on egg production was deduced by King (1961) from larger dam than sire components in an analysis of variance. Beilharz and McDonald (1961) reciprocally crossed Leghorn and Australorp breeds and reported maternal

line effects on egg production. Similar results were reported by Van Vleck et al. (1963). According to Hazel and Lamoreux (1947), 5 percent of the variation of 32 weeks body weight in Leghorns were accounted for by maternal effects, but no effect could be detected on sexual maturity. King (1961) and Van Vleck et al. (1963) reported significant maternal effects on 8 and 32 weeks body weight and on age at first egg.

Cock and Morton (1963) hypothesized that general size is controlled by maternal effects whereas sex-linked effects alters conformation, i.e., the relation of shank length to body weight. No evidence was found for the transmission of maternal effects beyond the F_1 generation. That maternal effects operate solely through egg size was considered unlikely.

Heritability estimates for part-record rate of lay in chickens, reported by Saadeh et al. (1968), were 0.10 and 0.24 from estimates of sire and dam variance components, respectively; the values for the full year record were 0.12 and 0.27, respectively. The differences were explained by either maternal effects or dominance. Craig et al. (1969), from the same laboratory, reported evidence for maternal effects on 32 and 55 weeks egg size.

Nordskog and Hassan (1971) found an important maternal effect associated with egg size genes on hatchability. A 10

gram increase in egg size above the optimal size of 50 grams lowered hatchability 10.7 percent while a 10 gram decrease lowered it 3.9 percent, this indicates asymmetry in maternal effect. Hatching speed was mainly determined by egg size and other maternal factors with the actual genotype of the embryo being a minor influence.

According to Briggs and Nordskog (1973), the W sex chromosome may also influence age of sexual maturity, and may also carry genes for egg weight. On the other hand, significant maternal effects on egg production could not be detected.

As related to mortality

It is well established that progeny from a Leghorn male x heavy female cross (LxH) have higher laying-house mortality than the reciprocal cross (HxL). Warren and Moore (1956) reported laying-house mortality of 38.4 percent when the Leghorn was used as the male parent, compared to 20.8 percent for the reciprocal cross. The higher mortality in the LxH cross could be attributed to the Leghorn sex chromosome or to the maternal effect of the heavy breed. Nordskog and Phillips (1960) analyzed mortality data of 3 breed-types, Leghorn (L), Heavy (H), and Fayoumi (F), and the reciprocal crosses among them. The crossbred pullets containing the Leghorn sex chromosome had the highest adult mortality. The

LxH cross had 24.0 percent mortality compared to 13.5 percent for the HxL cross. Similar results were found in the case of Leghorn x Fayoumi. The LxF cross had 24 percent mortality compared with 15 percent for the reciprocal. These results suggest that the difference in adult mortality of reciprocal crosses involving the Leghorn breed, are due to Leghorn sex chromosome.

A different conclusion was reached by Kawahara (1960) with the Leghorn (L) and Barred Plymouth Rock (BPR). Total adult mortality was higher in the LxBPR compared with BPRxL. The main cause of the difference in mortality was visceral leukosis. The incidence of deaths from leukosis among the crosses was higher than that among the purebred maternal half sisters. Resistance and susceptibility to visceral leukosis seemed to be controlled by maternal factors and direct effects of genes.

Higher mortality in the LxH cross compared to the reciprocal was reported by Hutt (1962). He suggested that the sex-linked broodiness gene (Saeki, 1957) was responsible for lower mortality in HxL, and that this explained the role of the Leghorn sex chromosome in increasing adult mortality. The idea was that genes for broodiness are associated with higher adult viability. This could not be substantiated however, from the earlier study reported by Nordskog and Phillips (1960).

Another possible interpretation of the role of genetic factors in mortality was given by Allen (1962), based on backcross data from the Leghorn and the Rhode Island Red. He postulated that differences in survival rates and in the incidence of visceral lymphomatosis between lines and crossbreds are the result of an interaction between the sex chromosome and the "plasmon". The plasmon was defined as "stable properties of the cytoplasma which may be transmitted to the offspring" such that its effect is transmitted from dam to daughter. When the sex chromosome and the plasmon are from the same source and the autosomes are heterozygotes, high viability results, but when they are from different breeds or even different strains within the same breed, higher mortality including an increase in visceral lymphomatosis results.

Morley and Smith (1951) compared mortality of reciprocal crosses between Leghorns and Australorps. Female progeny of the Leghorn male x Australorp female cross had higher mortality than the reciprocal cross. Morris and Skaller (1958) repeated this experiment but were unable to demonstrate statistically significant differences in mortality between the 2 reciprocal crosses. They suggested that favorable environmental conditions lower mortality in all the groups which might mask possible genetic differences. However, Beilharz and McDonald (1961), finding higher mortality in the progeny of Australorp hens, concluded that the mortality difference

between reciprocal crosses of Leghorns and Australorps resulted from a maternal breed effect.

A maternal effect on adult mortality was deduced by Moultrie et al. (1953). A high and a medium viable strain of Leghorns were reciprocally crossed; the adult mortality of the crosses differed little from that of their pure-line maternal half sisters. Mortality among the pure lines was mainly due to leukosis. The mortality of the cross-line pullets housed suggested a maternal influence on resistance to leukosis. A sex-chromosome effect on laying house mortality was reported by Morgan et al. (1972, Personal communication), working with both male and female progeny of Leghorn and Red reciprocally crossed. Only the reciprocal cross female progeny differed in mortality. This was taken as substantiating evidence that the sex chromosome affects adult mortality.

Briggs and Nordskog (1973) using a special experimental design, were unable to demonstrate a sex chromosome effect on mortality in reciprocal crosses of Fayoumi and a heavy-breed line. This was in agreement with an earlier study (Nordskog and Phillips, 1960). Thus. it seemed that the Fayoumi sex-chromosome had no effect on mortality. Moreover, a W sex-chromosome effect could not be detected. The maternal effect on mortality was large but not statistically significant.

A significant maternal effect on Marek's disease was reported by Han et al. (1969). Using the Brown Leghorn as the female parent, mortality in the cross was 20 percent higher than when other Leghorn lines were used as the female parent. These workers speculated that a susceptibility factor is transferred by the dam, either through an extra nuclear influence or by the W sex chromosome. Also it is possible that virus or antibodies are transmitted from dam to offspring through the egg.

B Blood Groups

At least 11 independently inherited blood group systems are known in chickens. The B system with many alleles has adaptive value (Gilmour, 1970). The apparent association of the B blood group with hatchability was first reported by Briles et al. (1953) from data on 3 inbred lines. The hatchability of heterozygous embryos was higher than that of homozygous embryos. The percentage of hatchability of the pure inbred lines averaged 53.6 (Briles and Krueger, 1955), and that of the heterozygous embryos of the crosses averaged 68.9. Chick livability to 9 weeks of age was 8-15 percent higher among the progeny of matings expected to produce 50 percent heterozygotes.

Briles et al. (1953) studied the B blood group effect on body weight in inbred lines of chickens. The heterozygous

cockerels were 7-10 percent heavier at 9 weeks of age than the homozygotes in 2 of the 3 lines. Body weight at 10 weeks was also found to be increased by heterozygosity (Briles, 1957).

A continuous segregation of 3 blood group loci was reported by Gilmour (1954). Later on he extended his findings to 7 independent blood group loci still segregating after 14 generations of brother-sister matings. He speculated a selective advantage for the heterozygotes (Gilmour, 1958, 1959).

Further results reported by Briles (1954, 1956a) seemed to substantiate the idea of heterozygous superiority. Hatchability of fertile eggs, representing 0, 50, 75, and 100 percent heterozygosity at the B locus, was 46, 62, 71, and 78 percent, respectively. The probability of an egg hatching from a heterozygous dam was 1.7 times greater than from a homozygous dam. Average egg production was 9-30 percent higher in the heterozygotes comparing to homozygotes.

Krueger et al. (1956) reported that livability was influenced by the B blood group system. Progeny of heterozygous males and noninbred females showed 2 percent better livability and 5 percent higher egg production than progeny sired by homozygous males. According to Briles (1956b) heterozygous females had higher egg production than the homozygotes in 2 inbred lines. No effect of the B genotype

on age of first egg could be detected.

In the 1950's the work of Briles and others was taken as evidence for an association between the B locus and economically important traits as egg production, fertility, hatchability, livability, and body weight. The heterozygotes seemed to be at an advantage (Gilmour, 1960; Briles, 1960).

High laying house mortality was reported for the B^1B^1 genotype, but not during the growing period (Briles and Allen, 1961). In a reciprocal recurrent selection experiment reported by Okada and Matsumoto (1962), aimed at the improvement of egg production using 2 inbred lines with 5 B alleles in each, natural and artificial selection appeared to have favored the heterozygous genotypes. The observed frequency of heterozygous genotypes in each population was higher than the expected frequency, and it further increased following selection of breeders.

Nordskog (1964) suggested that fitness genes may be linked with neutral B blood group genes such that there is apparent adaptive value of the blood group alleles. To further investigate this possible linkage, a randombred population might be useful because it would be in genetic equilibrium while in inbred lines, linkage equilibrium between fitness and blood group genes would not exist. Because it is difficult to develop specific blood typing reagents in a randombred population, an alternative is to synthesize a

population from well-typed inbred lines and so simulate a randombred population but with segregation for specific blood group alleles.

An influence of B blood group alleles on susceptibility to Marek's disease was reported by Hansen et al. (1967). Known B genotypes were both naturally and artificially exposed to the disease. Two types of inbred hybrids were produced, one containing B^{19} and the other containing B^{21} allele. A total of 854 chickens were inoculated with 0.1 ml inoculum taken from infected chickens; mortality data were recorded to 8-12 weeks of age. Chickens carrying the B^{19} allele had 29.0 percent mortality while those carrying B^{21} allele had only 16.0 percent mortality due to Marek's disease. In field experiments the B^{19} and B^{21} carriers had 12.4 percent and 6.5 percent mortality, respectively, in locations with a high incidence of Marek's. The B blood group genotypes of embryos affected embryonic mortality in 2 breeds of chickens, according to Gilmour and Morton (1970). Differences between genotypes of the dam were not significant.

Nordskog et al. (1973) proposed 2 alternative interpretations to explain the association of the B blood group locus with physiological processes in the fowl: 1) B genes are neutral but linked to fitness genes, and 2) B genes are accompanied by B modifiers whose pleiotropic effect is changed by natural selection. In their study 3 inbred lines with

known B alleles were used to synthesize 2 populations, S1 and S2, with 4 B alleles in each. Egg production and adult mortality of the 10 genotypes of each population were recorded over 5 years. The heterozygotes in the S1 population were superior to the homozygotes but this was due solely to the very poor performance of the B^1B^1 genotype. Mortality of the B^1B^1 was 54 percent compared to 10 percent for the B^1 heterozygotes and 6.8 percent for the non-B^1 genotypes. Similar results were found for egg production: 40.4, 53.4, and 56.0 percent respectively for B^1B^1, B^1B^x, and the non-B^1 genotypes. The B^1 allele was not introduced into S2 line, and in this line no statistical differences between homozygotes and heterozygotes could be detected.

An interesting observation in the above study was the apparent trend in improvement of the B^1B^1 genotype in both viability and egg production over successive generations. Adult mortality decreased an average of 4.4 percent per generation. This can be accounted for on the basis of the 2 hypotheses already mentioned. The first is that natural selection for modifier genes improved B^1B^1. Because natural selection would operate only on homozygotes and also because only 1/8 of the dams were B^1B^1 genotypes, natural selection would be very weak. Hence, it would seem that this would be an unlikely explanation for the improvement of B^1B^1 over generations.

The second and more tenable hypothesis would be that the B locus is neutral and linked to a fitness locus. Assuming that recombination takes place and given that the proportion of B^1B^1 genotypes in the population is constant each generation, the B^1B^1 genotype is expected to improve. Improvement would progress as linkages break up between the B locus and fitness locus linked to it and with natural selection then discarding the bad fitness gene.

Leukosis/sarcoma Viruses

The genetic mechanism involved in resistance to the leukosis/sarcoma viruses can be described in terms of a first and a second line of defense (Crittenden et al., 1972). The first is cellular resistance, i.e., the prevention of viruses from penetrating the living cells of the host.

The avian leukosis/sarcoma viruses have been classified into 5 subgroups A, B, C, D, E. Crittenden et al. (1967) reported 2 independent loci, tra and trb, which control cellular resistance to subgroups A and B, respectively. Two alleles at each locus were found in inbred Leghorn lines: a recessive allele for resistance and a completely dominant allele for susceptibility. The homozygous dominants and heterozygotes produced complete cellular susceptibility but the homozygous recessives were resistant to both A and B viral

infection.

A survey of commercial stocks (Crittenden and Motta, 1969) revealed that the white-egg producing stocks were highly susceptible to subgroup A, but they varied considerably for B subgroup (presumably due to gene segregation). Samples of heavy breeds were highly variable to A but resistant to the B subgroup. A third locus, C, was reported in a stock of inbred lines (Payne and Biggs, 1970) and in a noninbred flock of New Hampshire Chickens (Motta et al., 1973). The C locus appears not to be linked with tra and trb, but controls cellular resistance to subgroup C in the same way that tra and trb control resistance to the A and B subgroups.

The second line of defense is the genetic resistance against a leukosis/sarcoma virus which comes into play after the virus has already penetrated the cell (Crittenden, 1968). There may be a cellular interference in formation of viral progeny, or the host cell may develop either cellular or humoral immunity via the bursa of Fabricius. The actual genetic mechanism of control is undefined; its basis seems to be polygenic and confers resistance to leukosis sarcoma viruses in chickens lacking cellular resistance.

MATERIALS AND METHODS

Sex Chromosome Study

Description and development of lines

Two populations, different in performance and sex-linked traits were used in this study. Line 1 or GL is a Leghorn with colored plumage, i.e., it contains the nondominant white gene i and is small in body size. The sex chromosomes are designated Z_1 and W_1. Line 2 or H[1] is a heavy breed line synthesized from a cross between the Rhode Island Red and the Barred Plymouth Rock. It is large in body size and has gold-barred plumage. Its sex chromosomes are designated Z_2 and W_2, (Briggs, 1971). From these lines and crosses between them two 2x2 diallel sets were produced.

The first set, Y, consists of progeny from the pure lines GL and H and their reciprocal crosses GxH and HxG. The male line is indicated on the left and female on the right of the cross. For simplicity, Leghorn GL when used in a cross, is designated G.

The second diallel set, X, consists of 4 lines designated G^nH, H^nG, G^nX, and H^nX. The superscript, n indicates the generation number starting with 1968 as the 0 generation.

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[1]Line 2 or H is the same line W described by Briggs and Nordskog (1973). However, the designation, H, is used here in order not to confuse it with the sex chromosome symbol, W.

Only G^nH and H^nG were used in the analysis. The other 2 were used in the breeding plan starting in 1973.

The autosomal makeup of the Leghorn line is indicated by a ratio (100:0); meaning that the autosomes are 100 percent GL and 0 percent H. That for the heavy line is indicated by (0:100); meaning that the autosomes are 0 percent from GL and 100 percent from H. When the genotype contains equal proportions of autosomes from each parental line the designation is (50:50). The quantitative values of set X are designated by X_{11}, X_{12}, X_{21}, and X_{22} and those for set Y by Y_{11}, Y_{12}, Y_{21}, and Y_{22}. The autosomal makeup of all X groups is (50:50). The genetic makeup of the diallel sets are presented in Table 1.

The 2 lines GL and H, and their reciprocal crosses were produced each year, except 1973, in 2 shifts. In order to produce the pure lines, 6 Leghorn males were pen-mated to 80-90 Leghorn females and likewise 6 H males were mated in a separate pen to 80-90 H females. After 2 weeks the males were switched between the pens to produce the reciprocal crosses GxH and HxG. No males were kept in the pens for 4 days between shifts.

Only 2 of the 4 lines of the diallel set X were required for the analysis in this study. These were X_{12} with the genetic makeup, Z_1Z_1 (50:50) for the males and Z_1W_2 (50:50) for the females and X_{21} with the genetic makeup, Z_2Z_2 (50:50)

Table 1. Test populations in diallel sets X and Y (the dot subscript denotes a summation)

Diallel set Y

	Dams[a]		
	GL	H	
Sires[a]	Z_1W_1(100:0)	Z_2W_2(0:100)	
GL	Y_{11}(GL)	Y_{12}(GxH)	$Y_{1\cdot}$
Z_1Z_1(100:0)	Z_1W_1(100:0)	Z_1W_2(50:50)	Z_1(75:25)
H	Y_{21}(HxG)	Y_{22}(H)	$Y_{2\cdot}$
Z_2Z_2(0:100)	Z_2W_1(50:50)	Z_2W_2(0:100)	Z_2(25:75)
	$Y_{\cdot 1}$	$Y_{\cdot 2}$	$Y_{\cdot\cdot}$
	W_1(75:25)	W_2(25:75)	μ(50:50)

Diallel set X

	Dams		
	HxG	GxH	
Sires	Z_2W_1(50:50)	Z_1W_2(50:50)	
$G^{n-1}H$	$X_{11}(G^nX)$	$X_{12}(G^nH)$	$X_{1\cdot}$
Z_1Z_1(50:50)	Z_1W_1(50:50)	Z_1W_2(50:50)	Z_1(50:50)
$H^{n-1}G$	$X_{21}(H^nG)$	$X_{22}(H^nX)$	$X_{2\cdot}$
Z_2Z_2(50:50)	Z_2W_1(50:50)	Z_2W_2(50:50)	Z_2(50:50)
	$X_{\cdot 1}$	$X_{\cdot 2}$	$X_{\cdot\cdot}$
	W_1(50:50)	W_2(50:50)	μ(50:50)

[a] The sex chromosome constitution is indicated by Z (or W) and the autosome composition by the percentage proportion shown in parentheses. See text.

and Z_2W_1 (50:50) for males and females, respectively. The formation of the 2 lines is shown in Figure 1 and the sex chromosome makeup of the genotypes used in synthesizing them are given in Table 2.

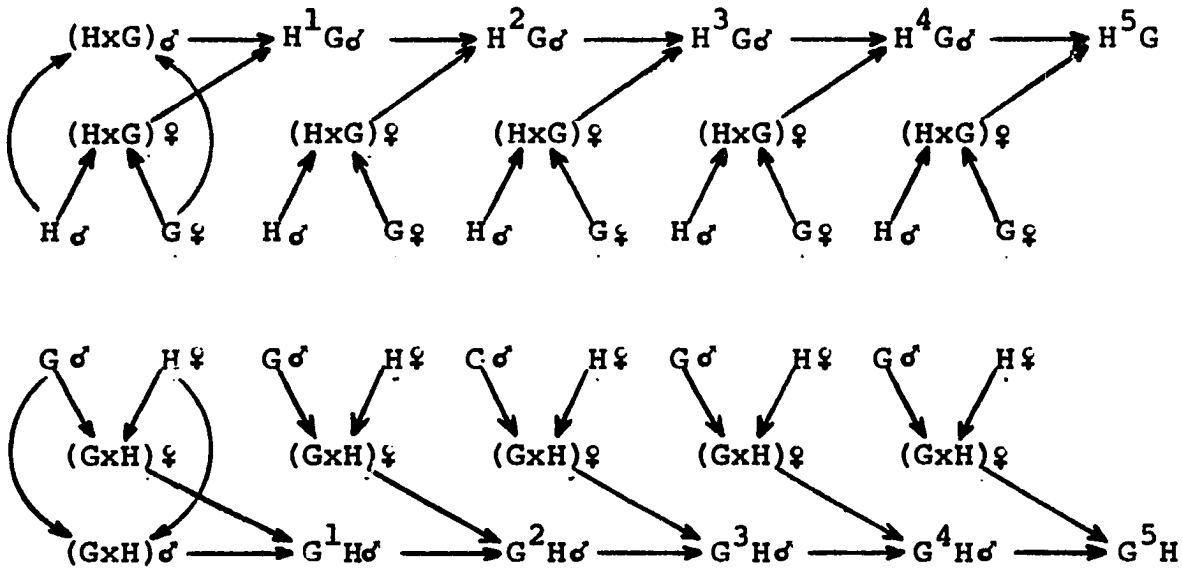
In 1969, an F_2 mating of the GxH cross was the first generation designated G^1H. Each Z sex chromosome in the sperm cells of the male parent, GxH, was expected to contain equal parts of Z_1 and Z_2 due to crossing over. The expected percentage of Z_1 in G^1H is 75 in the males and 50 in the females. G^1H males and GxH females were then used as breeders to produce the second generation G^2H. The expected Z_1 content would be 87.5 percent in the males and 75 percent in the females in the second generation. The G^3H males hatched in 1971 were mated back to GxH F_1 females to produce the G^4H population (1972). The H^4G line was synthesized in the same way, repeatedly crossing the H^nG males to HxG females.

The H^nG and G^nH are called backcross lines because they were crossed every generation to the F_1 females HxG and GxH, respectively. This was done in order to introduce the Z_1 chromosome of GxH into G^nH and the Z_2 chromosome of HxG into the H^nG line.

In this study the females G^nH and H^nG produced in 1971 and 1972 were contrasted with females of the pure lines and their reciprocal crosses. The sex chromosome content of the

Hatch year		1968	1969	1970	1971	1972	1973
Expected % of Z_2 sex chromosome	♀	100.00	50.00	75.00	87.50	93.75	96.87
	♂	50.00	75.00	87.50	93.75	96.87	100.00

Mating[a] type



Expected % of Z_1 sex chromosome							
	♂	50.00	75.00	87.50	93.75	96.87	100.00
	♀	100.00	50.00	75.00	87.50	93.75	96.87

[a] BC Backcross, F_1 cross, Po parental.

Figure 1. Development of G^nH and H^nG lines

Table 2. Sex chromosome makeup of the G^nH and H^nG lines

Line or cross designation		From mating ♂ x ♀	Sex chromosome composition in % ♂	♀
H	H^1G	(HxG)x(HxG)	75.00	50.00
	H^2G	H^1G x (HxG)	87.50	75.00
	H^3G	H^2G x (HxG)	93.75	87.50
	H^4G	H^3G x (HxG)	96.87	93.75
	H^5G	H^4G x (HxG)	100.00	96.87
G	G^1H	(GxH)x(GxH)	75.00	50.00
	G^2H	G^1H x (GxH)	87.50	75.00
	G^3H	G^2H x (GxH)	93.75	87.50
	G^4H	G^3H x (GxH)	96.87	93.75
	G^5H	G^4H x (GxH)	100.00	96.87

females in each backcross line was assumed to be 100 percent Z_1 or 100 percent Z_2 for the genetic analysis. Further details as to the role of G^nH and H^nG in the experimental design will be given in the section on statistical procedures. In 1973 all the lines of X and Y diallel sets were produced in 1 shift in 8 mating pens.

Statistical methods

The statistical model for the observed mean of progeny from one subtype following Briggs and Nordskog (1973) is,

$$Y_{ij}, X_{ij} = \mu + b_{aij}a + b_{hij}h + b_{zij}z + b_{mij}m + e_{ij}$$

where,

Y_{ij} = mean value of progeny from male line i and female line j in diallel set y

X_{ij} = the same as for Y_{ij} except that only groups X_{12} and X_{21} were available.

μ = mean of diallel set

a = Effect of line difference in autosomal genome composition

h = heterosis advantage of the crossbred progeny over the midparents

z = effect of line difference in Z sex chromosome composition

m = effect of maternal difference between lines

e_{ij} = sampling error due to replication of cell means over years and within cells. The errors were considered to be independently and normally distributed with mean zero

The coefficients for the parameters a, h, z, and m for diallel set Y are indicated in Table 3.

The a, h, z, and m effects were assumed fixed so that the coefficients of each parameter sum to zero over the 4 cells of set Y. The expectation of the cell mean, Y_{22} is,

$$Y_{22} = \mu + a - 1/2h + 1/2z + 1/2m$$

Table 3. Matrix of coefficients for diallel set Y (the dot denotes a summation)

	Parameter				
	μ	a	h	z	m
Cells					
Y_{11}	1	-1	-1/2	-1/2	-1/2
Y_{12}	1	-	1/2	-1/2	1/2
Y_{21}	1	-	1/2	1/2	-1/2
Y_{22}	1	1	-1/2	1/2	1/2
Margins					
$\bar{Y}_{1\cdot}$	1	-1/2	-	-1/2	-
$\bar{Y}_{2\cdot}$	1	1/2	-	1/2	-
$\bar{Y}_{\cdot 1}$	1	-1/2	-	-	-1/2
$\bar{Y}_{\cdot 2}$	1	1/2	-	-	1/2

The coefficients for the X_{12} and X_{21} subtypes are,

$b_{a12}=0$ $b_{h12}=0$ $b_{z12}=-1/2$ $b_{m12}=0$

$b_{a21}=0$ $b_{h21}=0$ $b_{z21}=1/2$ $b_{m21}=0$

The means of the 2 pure lines, GL and H, and their reciprocal crosses, in diallel set Y, contain variation due to autosomes, heterosis, sex chromosomes and maternal line effects. On the other hand, the 2 backcrosses G^nH and H^nG are expected to differ only in the Z sex chromosome.

Each of these has the same autosomal makeup so that there is no autosomal difference. Also there is no heterosis effect from the backcrosses which is defined only for the F_1 cross between the pure lines.

The dams of X_{12} and X_{21} are the Y_{12} and Y_{21} females, respectively, which do not transfer maternal lines effects to their progeny. The W sex chromosome effect remained confounded with the maternal line effect in order to allow estimation of the other parameters. It should be noted that Briggs and Nordskog (1973), using Fayoumi as the small body line, found no effect of the W sex chromosome on mortality.

In the diallel set Y, 3 separate combinations of parameters could be independently estimated. For the female progeny these are,

$$\text{sire line:} \quad (\bar{Y}_{2\cdot} - \bar{Y}_{1\cdot}) = \hat{a} + \hat{z}$$

$$\text{dam line:} \quad (\bar{Y}_{\cdot 2} - \bar{Y}_{\cdot 1}) = \hat{a} + \hat{m}$$

$$\text{interaction:} \quad 1/2(\bar{Y}_{21}+\bar{Y}_{12}) - 1/2(\bar{Y}_{11}+\bar{Y}_{22}) = \hat{h}$$

Parameter estimations were based on means, pooled over years, of the Y diallel set lines and the 2 backcrosses X_{12} and X_{21}. The estimates are,

$$\hat{z} = \bar{X}_{21} - \bar{X}_{12}$$

$$\hat{a} = (\bar{Y}_{2\cdot} - \bar{Y}_{1\cdot}) - (\bar{X}_{21} - \bar{X}_{12})$$

$$\hat{m} = (\bar{Y}_{\cdot 2} - \bar{Y}_{\cdot 1}) - [(\bar{Y}_{2\cdot} - \bar{Y}_{1\cdot}) - (\bar{X}_{21} - \bar{X}_{12})]$$

$$\hat{h} = 1/2(\bar{Y}_{21} + \bar{Y}_{12}) - 1/2(\bar{Y}_{11} + \bar{Y}_{22})$$

The $\hat{z}$, estimated from the difference between $\bar{X}_{21}$ and $\bar{X}_{12}$, was, in turn, used to obtain estimates $\hat{m}$ and $\hat{a}$ in diallel set Y assuming that the Z sex chromosomes have the same effect in either the X and Y lines.

An analysis of variance for diallel set Y was calculated. Mean squares were estimated for years, sire lines, dam lines, and sire by dam interaction (Table 4). Years were considered random while sire and dam lines were considered fixed effects. The significance of the variance components was tested against the error mean square using the F test. The error mean square was the pooled 2 and 3 way interactions of years sire type and dam type. The sire type mean square contains variation due to Z sex chromosome and autosomal differences. The dam mean square represents maternal and autosomal differences. A significant sire type by dam type mean square indicates heterosis. Separate analyses of variance were calculated for total laying house mortality and for each disease class.

Table 4. Analysis of variance of Y diallel set

Source	d.f.	Expected mean square
Year	1	$\sigma_e^2 + 4\sigma_Y^2$
Sire line (S)	1	$\sigma_e^2 + 4\sigma_S^2$
Dam line (D)	1	$\sigma_e^2 + 4\sigma_D^2$
SxD	1	$\sigma_e^2 + 2\sigma_{SD}^2$
Error	3	σ_e^2

The variances of parameter estimates are,

$$V_{\hat{z}} = V(\bar{X}_{21}-\bar{X}_{12}) = \frac{2\sigma_x^2}{2} = \sigma_x^2$$

$$V_{\hat{a}} = V[(\bar{Y}_{2\cdot}-\bar{Y}_{1\cdot})-(\bar{X}_{21}-\bar{X}_{12})] = \frac{\sigma_y^2}{4}+\frac{\sigma_y^2}{4}+\frac{\sigma_x^2}{2}+\frac{\sigma_x^2}{2} = \frac{\sigma_y^2}{2} + \sigma_x^2$$

$$V_{\hat{m}} = V(\bar{Y}_{\cdot 2}-\bar{Y}_{\cdot 1}) - [(\bar{Y}_{2\cdot}-\bar{Y}_{1\cdot})-\bar{X}_{21}-\bar{X}_{12})] = \frac{2\sigma_y^2}{4} + \frac{2\sigma_y^2}{4} + \frac{2\sigma_x^2}{2}$$

$$= \sigma_y^2 + \sigma_x^2$$

$$V_{\hat{h}} = V[1/2(\bar{Y}_{21}-\bar{Y}_{12})-1/2(\bar{Y}_{11}-\bar{Y}_{22})] = \frac{\sigma_y^2}{2}$$

σ_y^2 is the error estimate for a single group mean for diallel set Y obtained from the analysis of variance. The σ_x^2

is an error term for a group mean for diallel set X obtained from analysis of variance of H^nG and G^nH over 2 years.

Mortality data of these 2 lines were also analyzed by χ^2 test using 2x2 contingency tables (Snedecor and Cochran, 1967).

Autopsy procedures

The main purpose of this phase of the study was to detect possible associations of sex chromosomes with laying house mortality resulting from a specific disease. Laying house hen mortality for each line was recorded for 43 weeks commencing at housing in 1971 and 1972. Dead chickens were stored in a refrigerator at 4°C and taken weekly to the Veterinary Diagnostic Laboratory in Ames. The chickens were autopsied under the supervision of a veterinary pathologist. Cause of death was recorded according to the following classification.

1. Leukosis - there are 2 main categories; the first, Marek's disease (MD), is defined as a lympho-proliferative disease; it has also been called neural lymphomatosis or fowl paralysis. The disease is caused by a DNA herpesvirus group B. The classic form of the disease involves the peripheral nerves while the presence of visceral lymphoid tumors are usually associated with the acute form. Mortality from MD is highest at 10-20 weeks and may reach 60 percent.

The second is called the leukosis/sarcoma group. These diseases are caused by RNA myxoviruses. Under field conditions, the most common disease is lymphoid leukosis, known also as visceral lymphomatosis. The bursa of Fabricius, liver, spleen, and other visceral organs are primarily affected. The disease does not occur generally before 14 weeks of age. Peak of mortality occurs between 24 and 40 weeks (Hofstad, 1972). After 20 weeks it is very difficult to distinguish Marek's disease from lymphoid leukosis in gross postmortem examination because the gross lesions are highly similar. In 1971 and 1972 cases of lymphoid tumors were classified as "leukosis" and the disease was assumed to be lymphoid leukosis. This was justified, first on the basis that the test periods were between 20 and 63 weeks of age at which time the incidence of Marek's disease is usually low; secondly, all baby chicks were vaccinated against Marek's in 1972 which hopefully eliminated this disease from the experimental flocks.

2. Fatty liver syndrome - this condition may indicate a nutritional problem such as an excess of energy intake to output. However, the exact cause remains unknown. The bird usually shows signs of anemia, the combs and wattles are pale. Upon autopsy, the liver turns yellow

and mushy and is partly covered with blood clots from ruptured vessels. Fat depositions are found on visceral organs.

3. Diarrhea - this might be caused by a variety of pathogens and is frequently accompanied by emaciation and dehydration.

4. Enteritis and peritonitis - these symptoms may be caused by a variety of pathological situations.

5. Cannibalism - all cases of picking were classified under this syndrome. In some cases vent picking may have led to peritonitis but the case was classified only as cannibalism.

6. Reproductive disorders - these cases manifested involvement of the reproductive organs with various syndromes including internal layers, prolapse, ovarian dysfunction, salpingitis, and ovarian hemorrhages.

7. Other - certain disease conditions that may have caused death in a few cases including cutaneous tumors, starvation, coli-granuloma, hepatitis, airsacculitis, emaciation, dehydration, gizzard erosion, kidney involvement, and abdominal hemorrhages.

8. No diagnosis - in some cases no evidence cause for death could be found. Chickens which died of accidents during the laying period were not autopsied and were not considered part of the total laying house mortality.

Males were also autopsied during 1972, however, because the number of dead males at the end of the year was very small, mortality data were only analyzed by χ^2 test (Snedecor and Cochran, 1967).

B Blood Group Effects

Genetic stocks and autopsy procedures

The line used in this study was S1, synthesized in 1963 from 2 inbred lines (Rishell, 1968; Nordskog et al., 1973). Mortality data were collected on S1 in 1971 and 1972. Dead pullets were posted in an attempt to find the cause of death. Autopsy procedures and classification were the same as described previously in relation to the sex chromosome study. The S1 line was blood typed only at the B locus. The genotypes maintained were B^1B^1, B^1B^2, and B^1B^{19}. The main objective in collecting mortality data was to compare the disease resistance between the homozygote genotype B^1B^1 and the heterozygotes B^1B^2 and B^1B^{19}. Chi-square test was used to analyze the mortality data (Snedecor and Cochran, 1967).

Immune response to cellular antigen - Salmonella pullorum

An experiment was conducted to test for B locus differences in the immune response to Salmonella pullorum. In the spring of 1972, 20 adult hens of each of the 3 blood groups B^1B^1, B^1B^2, and B^1B^{19} were inoculated subcutaneously 3 times, 2 days apart, with a mixture of S. pullorum and bovine serum albumin (BSA).

S. pullorum bacterin was prepared according to standard procedures (Campbell et al., 1970). The suspension was diluted to contain approximately half a billion bacteria particles in a single injection of 0.5 ml.

The same experiment was repeated in the spring of 1973, using 15 adult hens of each of the blood groups B^1B^1 and B^1B^2. Each bird was inoculated with S. pullorum and ferritin. The bacterin was prepared according to the procedures used the previous year.

Blood samples were collected on the 7th, 14th, and 21st days in the 1972 experiment and on the 7th, 10th, and 14th days in 1973. The blood samples were allowed to stand in room temperature for a few hours, then placed in a refrigerator at 4°C overnight. The sera were harvested, placed in screw cap vials and stored at -20°C.

Anti S. pullorum agglutination titers were measured in the serum samples using the macroscopic tube agglutination

test (Campbell et al., 1970). Starting with 1:10 serum dilution, 10 serial 2-fold dilutions were prepared for each sample. To each of the tubes containing 0.5 ml of serum dilution, 0.5 ml of antigen was added. The tubes were first placed in a waterbath of 52°C for 4 hours, then in a refrigerator at 4°C overnight and read the morning after. The last dilution showing agglutination was considered to be the titer of that sample.

Serum samples obtained from the birds before inoculation were used as a negative control. Rabbit anti-S. pullorum immune serum was used as a positive control. An analysis of variance was calculated from $\log_{10}$ transformations of the agglutination titers to test for effects of genotypes and bleeding periods using a split plot design (Anderson and Bancroft, 1952).

Immune response to soluble antigens

Soluble antigens were added to the S. pullorum bacterin to test for differences in immune response of the various B genotypes to such mixtures. Each bird in the 1972 experiment was injected with 17.5 mg bovine serum albumin (BSA) per dose. In the 1973 experiment 7.5 mg ferritin per dose were injected into each bird in addition to the S. pullorum bacterin.

Bovine serum albumin Titration of the anti-BSA antibodies was accomplished by the passive hemagglutination technique with tanned sheep erythrocytes. By treating the erythrocytes with tannic acid and coating with BSA, they could be agglutinated by specific anti-BSA antibodies. Sheep red blood cells were washed in saline and a 2.5 percent suspension was prepared in buffered saline at pH of 7.2. Equal volumes of erythrocytes and 1:20,000 tannic acid were incubated for 10 minutes at 37°C. The suspension was centrifuged, the cells were again washed and finally resuspended to the original volume of the erythrocytes.

A BSA solution was prepared by adding 1 ml of BSA solution (50 µg per ml) to 4 ml of phosphate buffered saline at a pH of 6.4. One ml of tanned erythrocytes was added to the 5 ml protein solution and allowed to remain for 10 minutes at room temperature. The BSA coated, tanned erythrocytes were centrifuged and washed with 1 percent inactivated normal rabbit serum and absorbed with sheep erythrocytes. Finally they were resuspended in 1 ml of the rabbit serum. The serum samples to be tested were inactivated by heating for 30 minutes at 56°C. Preliminary tests showed that it was not necessary to absorb the samples with sheep erythrocytes.

Each sample was 2-fold diluted in 1:100 normal rabbit serum starting with a 1:10 dilution. A drop of BSA coated tanned erythrocytes was added to 0.5 ml of serum dilution, and

the tubes were allowed to set 3 hours at room temperature. The presence of antibodies in the sample was indicated by agglutinated cells spread over the entire bottom of a tube. A negative reaction was indicated by the cells settling at the bottom center of the tube. The titer of a sample was the last dilution showing presence of antibodies. Normal serum from the experimental hens served as a negative control and rabbit anti-BSA immune serum served as a positive control.

Ferritin Anti-ferritin antibodies were titrated with the passive hemagglutination technique with BDB-coupled erythrocytes. BDB (bisdiazobenzidine) is able to conjugate proteins to red cells. To 1 ml of ferritin solution containing 2000 mg ferritin, 8 ml saline, 1 ml phosphate buffer pH 7.3, and 1 ml of 5 percent washed sheep erythrocytes were added. A vial of BDB, kept at -20°C, was thawed and mixed with 7 ml cold phosphate buffer. To the ferritin erythrocytes suspension, 0.25 ml of the BDB solution was added and allowed to stay at room temperature for 10 minutes. The sensitized cells were centrifuged, washed with 3.5 ml of 1 percent inactivated normal rabbit serum and absorbed as described before. The cells were then resuspended with 2.5 ml of rabbit diluent. This amount of sensitized cells sufficed for titration of 5 samples with 16 dilutions in each sample.

The serum samples were inactivated at 56°C for 30 minutes

and absorbed with washed sheep red blood cells. Starting with 1:10 dilution, each sample was 2-fold diluted with the rabbit serum diluent. The samples were titrated as described for BSA. Titer values were statistically analyzed in the same way as with anti S. pullorum titers.

Immune response to viral antigen - parainfluenza 3 virus

An experiment was conducted in 1973 to test for B locus differences in immune response to a viral antigen. Fifteen adult hens of each of the blood types B^1B^1 and B^1B^2 were inoculated with commercial parainfluenza (PI) 3 vaccine.[1] Each pullet was injected intramuscularly in both thighs with 2 doses of 3/4 ml each. Blood samples were collected on the 7th, 10th, 14th, and 21st days. The sera were separated and kept at -20°C.

Using hemagglutination (HA) and hemagglutination inhibition (HI) microtiter tests, the samples were titrated for anti-PI 3 antibodies. Each serum was diluted 1:5 in saline and inactivated at 56°C for 25 minutes. Equal volumes of washed and packed bovine red blood cells and 0.02 g of acid washed kaolin were added to each sample to remove nonspecific agglutinins and to prevent cross reaction during the HI test. The samples were allowed to stand 10 minutes at room temperature and then separated from the kaolin and bovine cells. Bovine red blood cells for the HI test were washed 3 times

[1]Bar-3. Elanco Products Company, Indianapolis, Indiana.

in saline and diluted 1:4. Before a test, the suspension was further diluted 1:60. The PI 3 virus[1] was titrated using the HA test and diluted to give 4-8 HA units of antigen per 0.05 ml.

A drop of 0.05 ml of saline was placed in each well of a row on a microtiter plate except for the first. Quantities of 0.05 ml of serum sample were added to the first, second, and last well of the row. The 2-fold dilution was carried out beginning at the second well, using 0.05 ml loops. The last well of the row was not diluted and served as a serum control. Quantities of 0.05 ml of viral antigen were added to all wells except the serum control and allowed to react with the serum for 1 hour at room temperature. Similar quantities of 0.05 ml of bovine cells were added to all wells. The plates were placed at 4°C overnight and read the following morning.

A positive reaction was indicated when the red cells settled at the bottom center of the tube. Absence of antibodies was indicated by hemagglutination of the bovine cells by the viral antigen. Normal chicken serum and rabbit anti-PI 3 immune serum were used as negative and positive controls, respectively.

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[1]Kindly supplied by Dr. E. Jenney, U.S.D.A. National Animal Disease Laboratory, Ames, Iowa.

Cell Typing

The chorioallantoic membrane (CAM) inoculation technique was used to find the mode of inheritance to subgroups A and B leukosis/sarcoma viruses in GL and H lines. Purified Rous Sarcoma viruses (R.S.V.) stocks[1] for A and B subgroups were used in the assay. The A stock was designated BH-RSV (RAV-1) and the B stock BH-RSV (RAV-2).

Fertile eggs from the 2 lines were incubated under standard conditions. After the embryo was removed from the incubator on the 10th day, it was inoculated on the CAM with 0.2 ml of 1:100 virus A dilution or 0.2 ml of 1:10 virus B dilution. These dilutions of inoculum were each calibrated to give 100-200 pocks per susceptible CAM. The diluent was tris-buffered saline with 4 percent calf serum.

The inoculated embryos were then returned to the incubator; on the 18th day they were transferred to a refrigerator at 4°C and allowed to remain over night. The following morning, the chorioallantoic membranes were separated and the pocks were counted. The embryos were classified according to pock number per CAM: 0, 1-25, 26-50, 51-75, and 76+.

Two tests were performed in April, 1973. A total of

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[1]Kindly supplied by H. A. Stone, U.S.D.A. Regional Poultry Disease Laboratory, East Lansing, Michigan.

200 GL eggs and 200 H eggs were used in each test. Half of the embryos in each line were assayed for virus A and the other half for B.

RESULTS

Sex Chromosome and Disease Resistance

The number of pullets housed at the beginning of each test period as well as the number of posted pullets is presented in Table 5. Averages of laying house mortality are shown in Tables 6 and 7 and analyses of variance of mortality data are presented in Table 8.

Estimates of parameter differences between the Leghorn and heavy line populations were calculated in percentages (Table 9). Chi-square tests of contingency tables are presented in Table 10.

Laying house mortality

Total laying house mortality is presented in Tables 6 and 7. The GxH cross had consistently higher mortality than the reciprocal cross, 40.9 versus 27.3 percent, on the average, with the difference statistically significant ($P<0.05$). Mortality in the heavy pure line was higher than that of the light pure line in 1971 but somewhat lower in 1972.

Mortality was lower in all the lines of set Y in 1972 than in 1971. In 1972 the average mortality level in the GL, H, and HxG groups was 13-14 percent compared with 25.1 percent in the GxH group.

The lines of set X were consistently lower in mortality than those of set Y. Mortality in H^3G and H^4G was higher than that of G^3H and G^4H. The difference between the 2 set X lines was highly significant as indicated from the analysis of variance for total laying house mortality (Table 8). However, the χ^2 test of actual dead and alive pullets failed to detect any difference in rate of mortality between G^nH and H^nG groups (Table 10), indicating no Z chromosome effect on mortality.

The year effect was significant ($P<0.05$) with lower mortality in 1972. Higher mortality in the crosses than in the pure lines resulted in a sire line x dam line interaction ($P<0.10$).

Mortality of the males, as recorded in 1972, was very low except in the 2 reciprocal crosses (Table 5). There was no significant difference between GxH and HxG.

Total laying house mortality of females is evidently controlled by a complex genetic mechanism (Table 9). The heavy line maternal effect seems to be involved as well as autosomes of the light line and heterosis.

Leukosis

The average leukosis mortality was higher in the first year than in the second year of the study (Tables 6 and 7). In set Y leukosis accounted for 62.3 percent of the total laying

house mortality in 1971 and 55.8 percent in 1972. Similar decrease in mortality over years was recorded for set X. The difference in leukosis mortality is very probably accounted for by the vaccination against Marek's disease applied to all the baby chicks in 1972 for the first time. All the experimental flocks benefited from the vaccination. However, the improvement in viability was only 0.3 percent in the GxH cross.

Mortality from leukosis was higher in the GxH than in the HxG cross (Table 10). The difference was statistically significant ($P<0.05$). The difference in leukosis mortality between G^nH and H^nG was not significant indicating that the sex chromosome is not involved in leukosis mortality or more specifically, not in lymphoid leukosis.

Analysis of variance for leukosis mortality in females and parameter estimates are presented in Tables 8 and 9, respectively. The sire line effect was significant ($P<0.05$). The dam line effect was not significant ($0.05<P<0.10$). The heavy line maternal effect, autosomes of the Leghorn line and heterosis seem to be involved in leukosis mortality.

High mortality of the crosses, particularly GxH, relative to the pure line, resulted in a highly significant sire line x dam line interaction as well as a high value for heterosis ($\hat{h}$ = 15.8 percent).

Table 5. Number of birds housed and posted by years and genotypes

Disease classification	Genotypes											
	1971						1972					
	H	GL	HxG	GxH	H^3G	G^3H	H	GL	HxG	GxH	H^4G	G^4H
Females												
No. housed	243	129	133	118	135	176	158	176	165	175	160	162
Leukosis	33	14	35	52	7	10	10	11	12	34	8	7
Enteritis & peritonitis	2	1	3	4	2	-	3	3	1	3	3	2
Cannibalism	7	1	3	1	1	2	2	1	1	-	-	-
Reproductive disorders	2	-	1	1	1	1	1	3	1	2	2	2
Fatty liver	5	2	1	-	-	2	1	-	3	4	3	4
Diarrhea	7	3	3	4	1	-	1	6	-	-	1	-
Other	7	3	6	4	2	2	3	1	4	1	2	1
No diagnosis	3	-	3	1	2	1	-	-	-	-	1	1
Total Posted	66	24	55	67	16	18	21	25	22	44	20	17
Males												
No. housed	-	-	-	-	-	-	46	40	58	48	53	51
No. posted	-	-	-	-	-	-	2	4	15	15	3	4

Table 6. Pullet mortality in set Y (percent)

			1971			1972			Average		
			DAM			DAM			DAM		
			GL	H	MEAN	GL	H	MEAN	GL	H	MEAN
Total laying house mortality	SIRE	GL	18.6	56.8	37.7	14.2	25.1	19.6	16.4	40.9	28.6
		H	41.3	27.2	34.2	13.3	13.3	13.3	27.3	20.2	23.7
		MEAN	29.9	42.0	35.9	13.7	19.2	16.4	21.8	30.6	26.1
Leukosis	SIRE	GL	58.3	77.6	67.9	44.0	77.3	60.6	51.5	77.4	64.2
		H	63.6	50.0	56.8	54.5	47.6	51.0	59.0	48.8	53.9
		MEAN	60.9	63.8	62.3	49.2	62.4	55.8	55.0	63.1	59.0
Enteritis & peritonitis	SIRE	GL	4.1	5.2	4.6	12.0	6.5	9.2	8.0	5.8	6.9
		H	5.4	3.0	4.2	4.5	14.2	9.3	4.9	8.6	6.7
		MEAN	4.7	4.1	4.4	8.2	10.3	9.2	6.4	7.2	6.8
Cannibalism	SIRE	GL	4.1	1.3	2.7	4.0	0.0	2.0	4.0	0.6	2.3
		H	5.4	10.6	8.0	4.5	9.5	7.0	4.9	10.0	7.4
		MEAN	4.7	5.9	5.3	4.2	4.7	4.5	4.4	5.3	4.8
Reproductive disorders	SIRE	GL	0.0	1.3	0.7	12.0	4.3	8.1	6.0	2.8	4.4
		H	1.8	3.0	2.4	4.5	4.7	4.6	3.1	3.8	3.4
		MEAN	0.9	2.1	1.5	8.2	4.5	6.3	4.5	3.3	3.9

Table 6 (Continued)

			1971			1972			Average		
			DAM			DAM			DAM		
			GL	H	MEAN	GL	H	MEAN	GL	H	MEAN
Fatty liver	SIRE	GL	8.3	0.0	4.1	0.0	8.7	4.3	4.1	4.3	4.2
		H	1.8	7.5	4.6	13.6	4.7	9.1	7.7	6.1	6.9
		MEAN	5.0	3.7	4.3	6.8	6.7	6.7	5.9	5.2	5.5
Diarrhea	SIRE	GL	12.5	5.2	8.8	24.0	0.0	12.0	18.2	2.6	10.4
		H	5.4	10.6	8.0	0.0	4.7	2.3	2.7	7.6	5.1
		MEAN	8.9	7.9	8.4	12.0	2.3	7.1	10.4	5.1	7.7
Other	SIRE	GL	12.5	5.2	8.8	4.0	2.1	3.0	8.2	3.6	5.9
		H	10.9	10.6	10.7	18.1	4.2	11.1	14.5	7.4	10.9
		MEAN	11.7	7.9	9.7	11.0	3.1	7.0	11.3	5.5	8.4

Table 7. Pullet mortality in set X (percent)

Sex chromosome type	1971		1972		Average	
	G^nH	H^nG	G^nH	H^nG	G^nH	H^nG
Laying house mortality	10.2	11.8	10.5	12.5	10.3	12.1
Leukosis	55.6	43.7	41.2	40.0	48.4	41.8
Enteritis & peritonitis	0.0	12.5	11.7	15.0	5.8	13.7
Cannibalism	11.1	6.2	0.0	0.0	5.5	3.1
Reproductive disorders	5.5	6.2	11.7	10.0	8.6	8.1
Fatty liver	11.1	0.0	23.5	15.0	17.3	7.5
Diarrhea	0.0	6.2	0.0	5.0	0.0	5.6
Other	11.1	12.5	5.8	10.0	8.4	11.2

Table 8. Analysis of variance of mortality based on arcsin transformed percents (mean squares)

	Set Y					Set X	
Degrees of freedom Disease classification	Year 1	Sire line 1	Dam line 1	SxD 1	Error 3	Between Genotypes 1	Error 2
Total laying house mortality	336.7*	16.4	70.4	208.5***	24.6	2.4**	0.0
Leukosis	28.6	81.5*	50.6***	240.0**	6.8	28.3	4.6
Enteritis and peritonitis	55.9	0.5	0.7	13.6	18.9	202.8	48.8
Cannibalism	9.9	135.0**	3.6	97.5*	4.2	17.9	13.3
Reproductive disorders	134.2	1.7	0.0	2.3	33.1	0.0	8.3
Fatty liver	15.0	71.2	0.0	0.2	124.9	198.1	69.7
Diarrhea	74.8	41.6	44.0	377.1***	53.5	197.5**	0.1
Other	29.2	58.0	74.2	1.0	22.5	3.9	4.1

*$P<0.05$.

**$P<0.01$.

***$P<0.10$.

Table 9. Estimates of parameter effects (percents)

Disease classification	Parameter: Sex Chromosome $\hat{z}$	Autosomes $\hat{a}$	Maternal $\hat{m}$	Heterosis $\hat{h}$
Total laying house mortality	1.8	-6.7	15.5	15.8
Leukosis	-6.6	-3.7	11.8	18.1
Enteritis & peritonitis	7.9	-8.1	8.9	-3.0
Cannibalism	-2.4	7.5	-6.6	-4.3
Reproductive disorders	-0.5	-0.5	-0.7	-2.0
Fatty liver	-9.8	12.5	-13.2	0.9
Diarrhea	5.6	-10.9	5.6	-10.3
Other	2.8	2.2	-8.0	1.2

Table 10. Chi-square tests of mortality with one degree of freedom for each test year (number of birds)

Sex	Cross	Mortality from all causes						Leukosis pooled over years		
		1971			1972					
		Dead	Alive	Total	Dead	Alive	Total	Leukosis	Nonleukosis	Total
Females	GxH	67	51	118	44	131	175	86	25	111
	HxG	55	78	133	22	143	165	47	30	77
	Total	122	129	251	66	274	340	133	55	188
		$\chi^2 = 5.95^*$			$\chi^2 = 5.74^*$			$\chi^2 = 5.93^*$		
	G^n_nH	18	158	176	17	145	162	17	18	35
	H^n_nG	16	119	135	20	140	160	15	21	36
	Total	34	277	211	37	285	322	32	39	71
		$\chi^2 = 0.2$			$\chi^2 = 0.31$			$\chi^2 = 0.34$		
Males	GxH				15	33	48			
	HxG				15	43	58			
	Total				30	76	106			
					$\chi^2 = 0.73$					

*P<0.05.

Minor disease classes

Nearly 59 percent of the pullets autopsied were classified as dead from leukosis. The balance (41 percent) was classified into 6 other minor disease classes as already described. No single minor class accounted for more than 9.1 percent of the posted pullets; and none can clearly account for total laying house mortality of GxH compared with HxG cross.

Enteritis and peritonitis The number of mortality cases from enteritis and peritonitis accounted for less than 7 percent of the total posted pullets. In 1971 the difference between the reciprocal crosses was nil. Mortality of the pure lines was somewhat lower compared with the crosses (Table 5). On the other hand, in 1972, a higher incidence of enteritis and peritonitis was found in the pure lines than in the crosses. Mortality from these causes was 6.5 percent in GxH versus 4.5 in HxG cross (Table 6).

More cases of enteritis and peritonitis were found in 1972 than in 1971. Mortality in H^nG line was higher than in G^nH (Table 7).

Cannibalism Less than 5 percent of the posted pullets were diagnosed as cannibalism. The incidence was higher in the heavy line compared with the light line over the 2 years study. The heavy line had the highest mortality

in set Y. The incidence of cannibalism was consistently higher in the HxG than in GxH cross (Table 6). Higher mortality from cannibalism in G^nH than H^nG line was found in 1971. No pullets died from cannibalism in 1972 in either the G^nH or the H^nG lines.

The sire line effect from the analysis of variance was highly significant. The sire line x dam line effect was significant (Table 8). These seemingly statistically significant variance components may be a consequence of sampling errors due to the small number of pullets in some of the percentage values subjected to analysis of variance.

Reproductive disorders Very few pullets died from reproductive disorders, with no more than 3 cases per line per year. Average mortality was higher in 1972 than in 1971 for both the X and Y sets. The incidence of reproductive disorders was slightly higher in the HxG than in the GxH cross and in the G^nH than H^nG line (Tables 6 and 7).

Fatty liver The incidence of mortality from fatty liver syndrome was higher in G^nH than in H^nG, or 17.3 versus 7.5 percent, respectively (Tables 6 and 7). The incidence of fatty liver was consistently higher in HxG compared with the GxH cross. Differences between the pure lines and between the crosses were not significant.

Diarrhea The number of pullets dying from diarrhea as the posted cause was low. In 1972 only 8 pullets were recorded following autopsy (Table 5). Mortality in the pure lines was consistently higher than in the reciprocal crosses. No diarrhea cases were found in the GxH and HxG crosses in 1972.

A sire line x dam line interaction effect could be detected in the analysis of variance of set Y ($P<0.10$) but no other effect was significant.

Other The results classified as "other" (Tables 5, 6, 7, 8, and 9) are presented here only for completeness of the mortality study. Miscellaneous autopsy findings were lumped together in this class. The mortality in this classification was low in both sets X and Y and accounted for 9.1 percent of total laying house mortality.

Genetic parameters Estimates for genetic parameters were calculated for the minor disease classes and are presented in Table 9. In the case of diarrhea only 1 pullet died from diarrhea in each of the lines H^3G and H^4G but none in G^3H and G^4H. This seems to account for the "highly significant" genotype effect in the analysis of variance (Table 8) in addition to a Z_2 effect. Because of the small number of dead pullets in the minor classes, these results must be interpreted with caution. The analysis of variance

Table 11. Pullet mortality by B locus genotypes and years in the S1 line

Year	B^1B^1				B^1B^x			
	% Total mortality	No. posted	% leukosis	% diarrhea	% Total mortality	No. posted	% leukosis	% diarrhea
1972	33.3	11	27.2	18.1	14.5	13	23.0	38.4
1973	38.6	29	13.7	58.6	5.0	8	12.5	50.0
Mean	35.9	-	20.4	38.3	9.7	-	17.7	44.2

Table 12. Chi-square tests of pullet mortality in the S1 line pooled over years with one degree of freedom for each test (number of pullets)

Genotype	Total mortality			Leukosis mortality			Diarrhea mortality		
	Dead	Alive	Total	Leukosis	Non-leukosis	Total	Diarrhea	Non-diarrhea	Total
B^1B^1	40	105	145	7	33	40	19	21	40
B^1B^x	21	285	306	4	22	26	9	17	26
Total	61	390	451	11	55	66	28	38	66
	$\chi^2 = 36.12^{**}$			$\chi^2 = 0.05$			$\chi^2 = 1.05$		

$^{**}P<0.01$.

probably gives an underestimate of the true sampling error.

B Blood Group Effects

Laying house mortality

During the laying house period, 1/3 of the homozygous (B^1B^1) pullets died compared with 1/10 of the heterozygous (B^1B^x) pullets, the difference is statistically significant. The data from B^1B^2 and B^1B^{19}, not being significantly different, were pooled and designated B^1B^x. The mortality data and analysis are presented in Tables 11 and 12.

As to disease classes, only the data on the leukosis and diarrhea were analyzed because the posted deaths from other classes were small. Leukosis mortality was 20.4 percent in the B^1B^1 and 17.7 percent in B^1B^x group. Mortality from diarrhea was slightly higher in B^1B^x. Of the pullets autopsied, 38.3 percent in the B^1B^1 group and 44.2 percent in the B^1B^x group were classified as diarrhea cases. The differences were not statistically significant.

Cellular antigen - *Salmonella pullorum*

In general, antibody titers to *S. pullorum* declined after reaching a peak on the 7th day post inoculation (Table 13). In 1972 the average titer of the homozygous B^1 pullets was only 25 percent as high as that of B^1B^2 in the 1st week and 60 percent as high in the 2nd week. Also the

Table 13. Immune response of B blood group engotypes (mean titers)

Antigen	GENOTYPES														
	B^1B^1					B^1B^2					B^1B^{19}				
	Days post inoculation					Days post inoculation					Days post inoculation				
	4	7	10	14	21	4	7	10	14	21	4	7	10	14	21
S. pullorum 1972		172		58	20		682		90	32		404		85	35
S. pullorum 1973	9	180	106	57		37	328	258	80						
BSA		176		207	84		191		214	169		174		177	121
Ferritin	1933	4662	3520	741		2667	6811	5531	1130						
PI 3[a]		32	248	38	36		40	72	46	52					

[a]Hemagglutination inhibition units.

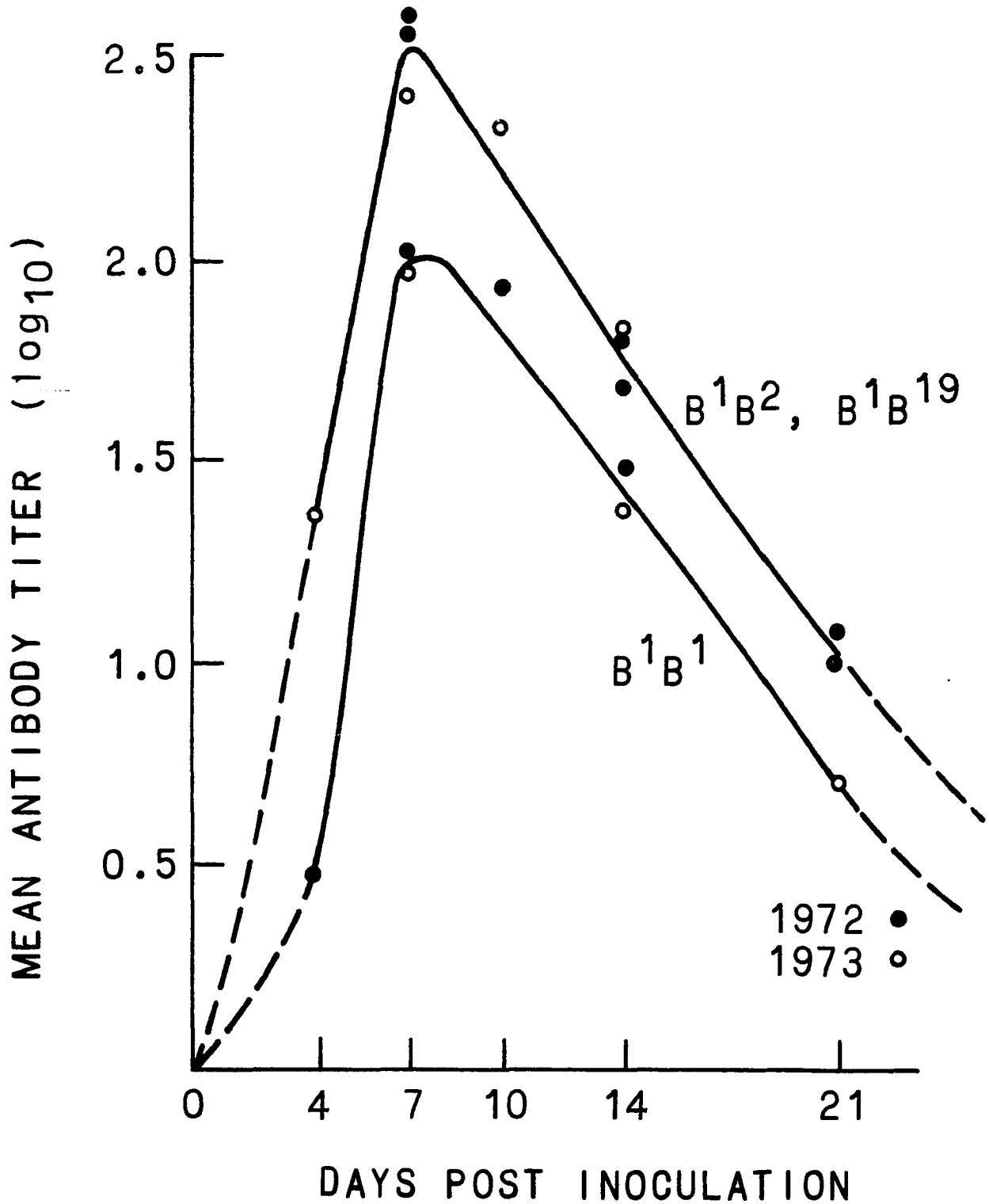


Figure 2. Immune response of genotypes B^1B^1, B^1B^2 and B^1B^{19} to S. pullorum

2 heterozygotes differed in their response. In the 1st week, the average titer of the B^1B^{19} was about 60 percent as high as that of B^1B^2, but the difference disappeared in the 2nd week.

The difference in response to S. pullorum was statistically significant in an analysis of variance (Table 14). The sum of squares for genotypes was divided into 2 orthogonal contrasts. The difference between the B^1 homozygotes and the average of the heterozygotes, B^1B^2 and B^1B^{19}, was high significant ($P<0.01$). On the other hand, the difference between B^1B^2 and B^1B^{19} was not statistically significant.

Superior antibody production by the heterozygotes was again observed in a second experiment in 1973. This time only the genotypes B^1B^1 and B^1B^2 were tested. The average titers of the homozygotes were 24, 54, 41, and 71 percent as high as that of B^1B^2 on the 4th, 7th, 10th, and 14th days post inoculation, respectively. The difference between the genotypes was highly significant.

The period effect was highly significant in both years as expected. Typically after the injection of an antigen the antibody level in the blood gradually increased and after reaching a peak, declined.

The significant genotype x period interaction ($P<0.05$) in 1973 indicates that the 3 genotypes have different curves of antibody production over time. In 1972, however, the

Table 14. Analysis of variance of immune response to S. pullorum (log_{10} of immune titers)

Source	1972 df	1972 M.S.	1973 df	1973 M.S.
Genotype	2	3.953*	1	7.736**
B^1B^1 vs. $(B^1B^2 + B^1B^{19})/2$	1	7.885**	-	-
B^1B^2 vs. B^1B^{19}	1	0.021	-	-
Pullets/genotype	57	0.899	28	0.528
Period	2	32.321**	3	10.472**
GxP	4	0.201	3	0.502*
Error	113	0.172	84	0.159

*$P<0.05$.

**$P<0.01$.

interaction was not statistically significant. A graphic picture of the immune response of B locus blood group genotypes to S. pullorum, monitored over time, is presented in Figure 2. The curve was fitted visually. Individual titers are given in the Appendix (Tables 19a and 19b).

Soluble antigens

Bovine serum albumin No significant differences in immune response between the B^1B^1, B^1B^2, and B^1B^{19} genotypes were found following stimulation with BSA (Tables 13, 15 and Figure 3). For all the pullets the response to this

antigen was weak (Appendix, Table 20). The titers increased slightly between the 7th and 14th days post inoculation and then declined. The average titers of B^1B^2 were somewhat higher than that of the B^1B^1. On the 21st day post inoculation, the average titer of the B^1B^1 pullets was 50 percent as high as the B^1B^2.

Ferritin Pullets of the 2 blood groups B^1B^1 and B^1B^2 were inoculated with the antigen in 1973. The results are presented in Tables 13, 15, and in Figure 3. Individual titers are given in the Appendix (Table 21).

The antibody titers were relatively high as early as the 4th day post inoculation and reached a peak on the 7th day. The higher capability of the B^1B^2 pullets to produce antibodies to ferritin was demonstrated over the entire experimental period. The average titers of the B^1B^1 pullets were 72, 68, 63, and 65 percent as high as the B^1B^2 pullets in the 4th, 7th, 10th, and 14th days post inoculation, respectively. On the other hand, a statistically significant genotype effect could not be demonstrated.

Viral antigen - parainfluenza 3 virus

The immune response of B^1B^1 as well as B^1B^2 pullets to PI 3 was very weak (Table 13). In the B^1B^1 group the average titer on the 7th day post inoculation was 32 hemagglutination inhibition (HI) units. It increased to a peak of

248 HI on the 10th day. Individual pullets results are presented in the Appendix (Table 22).

The average titer in the B^1B^2 group was initially higher (40 HI), reaching a maximum of 72 HI on the 10th day and then declined to a level somewhat higher than that of the B^1B^1. Differences between genotypes were not significant (Table 15, Figure 3).

Cell Typing

During this study GL and H embryos were inoculated with subgroup A and subgroup B avian leukosis viruses in 2 tests. Crittenden and Motta (1969) found significant heterogeneity among tests based on χ^2 test. They recommended that line comparisons be made only within tests.

A similar check was performed on the results obtained in this study (Table 16). In 2 cases χ^2 values were not significant and in the other 2 cases, χ^2 analysis indicated some heterogeneity ($0.025<P<0.05$). Based on the χ^2 analysis, the differences among tests were judged as chance effects. The pooled results of the 2 tests are presented in Table 17 and in Figure 4.

Both the GL and H lines are evidently still segregating for resistance to subgroup A virus. Nearly 55 percent of the GL embryos were completely resistant to the virus; 25 percent were completely susceptible. Few embryos showed

Table 15. Analysis of variance of immune response to bovine serum albumin, Ferritin, and Parainfluenza 3 virus (log_{10} of the immune titers)

Source	BSA		Ferritin		PI 3	
	df	M.S.	df	M.S.	df	M.S.
Genotype	2	0.473	1	0.703	1	0.393
Pullets/Genotype	56	0.469	28	0.546	28	1.078
Period	2	0.281	3	3.200**	3	1.082
GxP	4	0.017	3	0.040	3	0.741
Error	109	0.130	80	0.090	80	0.561

**$P<0.01$.

Figure 3. Immune response of genotypes B^1B^1, B^1B^2, and B^1B^{19} to ferritin, bovine serum albumin, and parainfluenza 3 virus

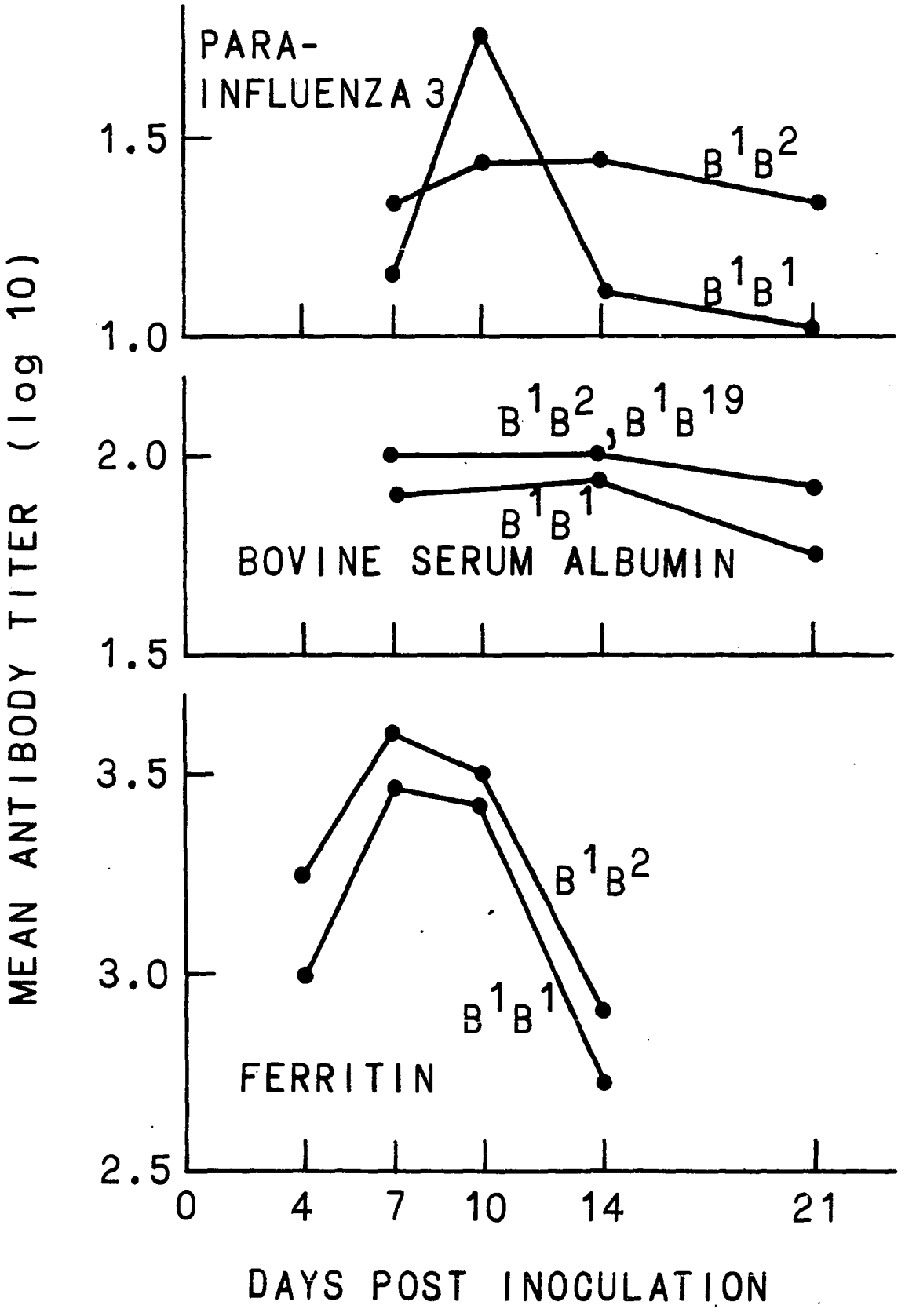

PARA-
INFLUENZA 3
1.5
1.0
B1B2
B1B1
2.0
B1B2, B1B19
B1B1
BOVINE SERUM ALBUMIN
1.5
3.5
3.0
B1B2
B1B1
FERRITIN
2.5
0
4
7
10
14
21
MEAN ANTIBODY TITER (log 10)
DAYS POST INOCULATION

Table 16. Distribution of pock counts on the chorioallantoic membranes of embryos inoculated with avian leukosis virus subgroups A and B (number of embryos)

Line	Test	Total embryos classified	Pock Counts 0	1-25	26-50	51-75	76+	χ^2
			Subgroup A					
GL	I	82	42	7	4	7	22	
	II	54	32	2	2	6	12	2.156
H	I	80	62	8	5	2	3	
	II	80	56	4	2	7	11	10.256*
			Subgroup B					
GL	I	75	46	6	5	2	16	
	II	79	30	14	4	4	27	10.001*
H	I	75	67	8	0	0	0	
	II	54	53	1	0	0	0	3.818

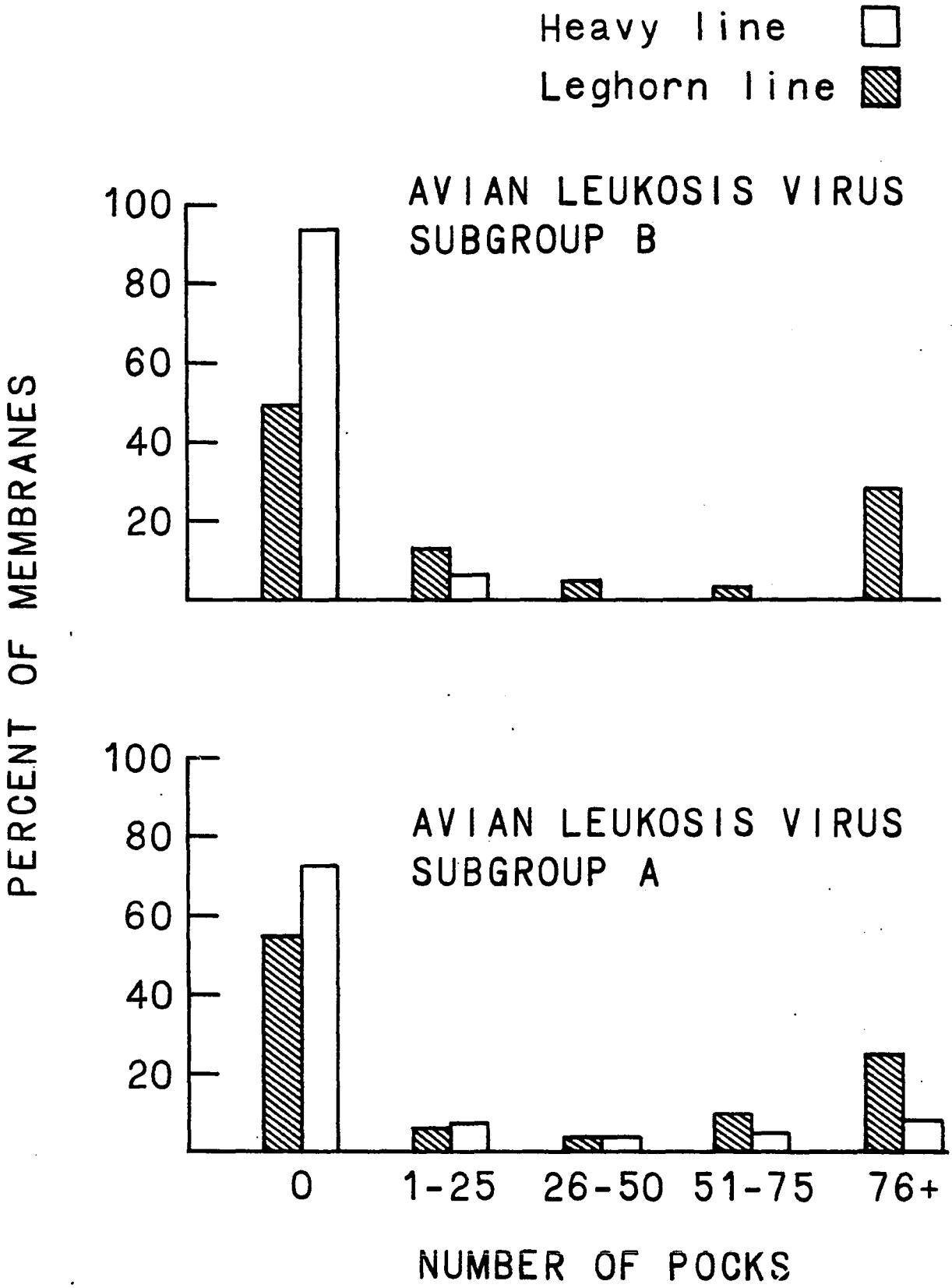
*$0.025<P<0.05$.

Table 17. Distribution of pock counts on the chorioallantoic membranes of embryos inoculated with avian leukosis virus subgroups A and B pooled over 2 tests

Virus subgroup	Line	Total embryo classified	Pock Counts 0	1-25	26-50	51-75	76+	χ^2
			Number of Embryos					
A	GL	136	74	9	6	13	34	
	H	160	118	12	7	9	14	17.504**
B	GL	154	76	20	9	6	43	
	H	129	120	9	0	0	0	70.620**
			Percentage of Embryos					
A	GL	136	54.4	6.6	4.4	9.5	25.0	
	H	160	73.7	7.5	4.3	5.6	8.7	
B	GL	154	49.3	12.9	5.8	3.8	27.9	
	H	129	93.0	6.9	0	0	0	

**$P<0.01$.

Figure 4. Response of Leghorn and Heavy line embryos to chorioallantoic membrane inoculation with avian leukosis virus subgroups A and B


Heavy line
Leghorn line
AVIAN LEUKOSIS VIRUS
SUBGROUP B
AVIAN LEUKOSIS VIRUS
SUBGROUP A
PERCENT OF MEMBRANES
100
80
60
40
20
0
1-25
26-50
51-75
76+
NUMBER OF POCKS

incomplete resistance or susceptibility.

The distribution of pocks in the H embryos indicated higher resistance to virus A. Nearly 74 percent of the embryos were resistant and 8.7 percent were susceptible. Chi-square tests indicated highly significant differences between the 2 lines. Line H is more resistant to virus A than line GL.

Greater resistance to virus B was found in line H compared with line GL. Most of the H embryos were completely resistant to virus B while less than 50 percent of GL embryos showed resistance to this virus. In general, the heavy line had higher cellular resistant to subgroup A and B viruses than the Leghorn line.

To obtain a broader perspective of genetic resistance to leukosis/sarcoma viruses, embryos of 7 ISU inbred lines were inoculated with A and B viruses. Pock distribution for each of these lines is presented in Table 18.

The Fayoumi line M and the Spanish line SP were completely resistant to the A and B viruses. Leghorn line 19 was susceptible to A but evidently is still segregating for the B subgroup. Leghorn line GH showed considerable variability for both subgroups A and B. Leghorn line HN varied for A but was susceptible to subgroup B. Leghorn line 9 still segregated, seemingly with a tendency for fixation of cellular resistance. Leghorn line 8 was variable for subgroup A but essentially resistant to subgroup B.

Table 18. Distribution of pock counts on the chroioallantoic membranes of embryos of 7 inbred lines inoculated with avian leukosis virus subgroups A and B (number of embryos)

Breed	Line	Fx	Total embryos classified	Pock counts				
				0	1-25	26-50	51-75	76+
				A subgroup				
Leghorn	L-19	0.97	27	1	1	0	7	18
Fayoumi	M	0.20	25	22	1	0	0	2
Spanish	SP	0.65	24	20	4	0	0	0
Leghorn	GH	0.58	25	11	1	0	2	11
Leghorn	HN	0.86	24	6	0	1	1	10
Leghorn	L-9	0.91	27	21	2	1	0	3
Leghorn	L-8	0.93	21	11	1	0	4	5
				B subgroup				
	L-19		20	8	1	1	2	8
	M		25	25	0	0	0	0
	SP		25	23	0	0	2	0
	GH		20	5	1	0	2	12
	HN		19	1	1	0	0	17
	L-9		23	15	3	2	1	2
	L-8		22	21	0	1	0	0

DISCUSSION

Basic Disease Resistance Mechanisms

The purpose of this section of the Discussion is to review briefly, known immune mechanisms and then to attempt to relate the observed experimental results from this study to what is already known with regard to genetic aspects of the immune response.

Immunity of an individual to an infectious disease agent depends upon mechanisms which: (1) prevent the penetration of a pathogen into the body, (2) inhibit or destruct pathogens in the body tissues after they have invaded the body and (3) eliminate toxic material liberated by pathogens in the body. Host immunity can be natural or acquired. Natural, or innate immunity, exists in an individual without previous exposure to an antigen. Acquired immunity is demonstrated after stimulation with an antigen. Natural and acquired immunity can protect the individual independently or by operating together.

Natural immunity

Natural immunity may sometimes imply an absolute resistance to certain diseases. For example, humans do not develop hog cholera; cold blooded animals are nonsusceptible to endotoxins. Usually, natural immunity is not absolute but relative.

Natural antibodies are blood substances which react specifically with invading antigens (Boyden, 1966). It is not clear yet whether these substances are under genetic control without previous contact with the antigen. Sprague (1958) reported that the capacity of cattle to produce natural antibodies to the blood group substance, Oc, is controlled by single dominant gene.

According to Weiser et al. (1969), there are external and internal anatomic and biochemical barriers which protect the host from penetration of pathogens. The body integument is normally populated by a flora of microorganisms which produce antimicrobial substances. Similar antibacterial activity is found in mucous secretions. The skin is a mechanical barrier to invasion of bacteria into the body. Organic acids produced by the normal flora of bacteria on the skin and unsaturated long-chain fatty acids, produced in the sebaceous glands, possess antimicrobial activity. The mouth, the nose, and the respiratory tract are covered with cilia which act as barriers against bacteria and viruses. Natural substances in the saliva, nasal, and respiratory tract secretions serve as protective agents. The bacteria which reach the stomach are destroyed by the gastric acids.

Physiologic barriers also provide protective mechanisms against pathogens. High body temperature inhibits the invading bacteria from growing. Anthrax bacilus can grow

only within a certain range of temperatures. It will not grow in chickens with a body temperature of 41°C but only when the body temperature drops to 37°C.

Certain animals do not provide the metabolites necessary for microbial multiplication. High oxygen tension, as found in the lungs, prevents the growth of anaerobic microorganisms. Phagocytes and macrophages destroy invading pathogens by engulfment and digestion. Tissue cells may have resistance to virus infection because they lack the specific surface receptors required for entry by the virus particle. Crittenden (1968) has described the significance of cellular resistance of chickens to avian leukosis viruses.

Certain enzymes, such as lysozyme, found in animal tissues and in egg albumin, may have bactericidal properties (Salton, 1957). Lysozyme is a muramidase and destroys bacteria particles by attacking muramic acid in the bacterial cell wall. Donaldson et al. (1964) described 3 substances found in human serum, β lysin, leukin, and plakin, which are capable of antimicrobial activity.

Complement is an important part of the immune mechanism and functions by interacting with other immune mechanisms (Muller-Eberhard, 1968). Other naturally occurring substances, such as interferon and properdin, also have antiviral and antibacterial activity.

The barriers to pathogens may be under specific genetic

control. For example, 2 independent loci reported by Lilly (1966), control susceptibility to the Gross leukemia virus in mice. The cellular resistance of chicken cells to leukosis/sarcoma viruses has already been mentioned. Independently segregating loci designated A, B, and C govern the ability of the corresponding virus subtypes A, B, and C to penetrate the chicken cell (Crittenden, 1968). Other cases of genetically determined host susceptibility to specific pathogens may be of a polygenic nature (Hildemann, 1970).

Acquired immunity

Acquired immunity is achieved by antigen stimulation of the immune mechanism to produce antibodies. The antibodies fight pathogens in several ways. For example, they neutralize toxins and viruses. Together with complement, they defend the body against certain gram negative bacteria. The joint action of antibodies and natural immunity provides a mechanism of disease resistance. Homograft rejection and delayed hypersensitivity are examples of acquired cellular immunity. These are mediated by cells and not by antibodies.

The thymus is an important organ of immune response. Miller (1962) found that mice, thymectomized at birth, were not fully immunocompetent. In particular, they were unable

to reject homografts and respond weakly to antigenic stimulation. This impairment of the immune mechanism has also been observed by Miller (1962) in thymectomized rats and chickens.

Glick et al. (1956) showed that the bursa of Fabricious is involved in the immune resonse. Bursectomized chickens failed to yield normally high level of antibodies to antigen stimulation. Cooper et al. (1966), in a series of experiments concluded that the bursa and the thymus in chickens are 2 distinct immune systems. The thymus-dependent small lymphocytes take part in cellular immunity such as the graft versus host response, delayed hypersensitivity and homograft rejection. Thymus-dependent lymphocytes are believed to play only a minor part in antibody response. The bursa-dependent cells, recognized as large lymphocytes and plasma cells, produce humoral or circulating antibodies.

According to Good (1972) the dual immune systems, may apply to mammals as well as the chicken. Stem cells, originating in the yolk sac of the embryo, migrate into the fetal liver, differentiate along several paths becoming hematopoietic elements. The lymphoid stem cells mature into 2 distinct populations. Those lymphocytes which mature in the thymus are called T cells and those which mature in some bursa-like organ are called B cells. However, it is

not yet clear what the bursal-equivalent organ in mammals really is. The bone marrow and the spleen evidently possesses bursal activity. Even in chickens, precursors of B cells have been found in the bone marrow and in the spleen.

The T cells take part in cellular immunity and also defend the body against certain viruses, fungi, bacteria, and malignant cells. The B cells produce antibodies, by first synthesizing peptide chains, assembling them into immunoglobulins, and secreting them. Approximately 70-80 percent of the lymphocytes in the circulation are T cells, the rest are B cells. Perhaps, under certain conditions, the T as well as the B system may respond to particular antigens (Good, 1972).

Sex Chromosome and Disease Resistance

The first part of the present study was designed to find the role that the sex chromosome plays in disease resistance. The difference in mortality between the 2 reciprocal crosses of the Leghorn line, GL, and the heavy line, H, might be accounted for by any one or a combination of the following: (1) a Leghorn sex chromosome effect, (2) a heavy-line maternal effect, (3) an autosomal effect and (4) heterosis. The female progeny of the 2 synthesized lines, G^nH and H^nG, were assumed to differ only with respect to the

Z chromosome. At the same time, the W chromosome was assumed to have no effect on mortality (Briggs and Nordskog, 1973).

The results clearly showed, in agreement with the literature, that total pullet mortality was significantly higher in the GxH than in the HxG cross. Yet, the difference between the corresponding synthetic lines, G^nH and H^nG, was small and nonsignificant. The evidence, therefore, indicates that the Z sex chromosome, by itself, is not the cause of the difference in adult mortality.

The difference in cockerel mortality between the 2 reciprocal crosses was small and not statistically significant. This was in agreement with Morgan (personal communication) who found a significant reciprocal cross difference in the female progeny mortality of Leghorns mated to Rhode Island Reds, but no difference in mortality among male progeny of the 2 reciprocal crosses. He interpreted his results as a direct effect of the Leghorn sex chromosome on adult mortality.

One possible explanation why the results of this study conflicts with Morgan's experiment might be that the genetic factors affecting mortality in females are not the same as in males. For example, the sex hormones may be involved leading to an interaction difference of sex and reciprocal crosses. This hypothesis could be checked by designing an experiment comparing hormone-treated females in a reciprocal

cross. At the same time, caponized males might be compared in a reciprocal cross to test for a maternal line effect. In any event, it seems clear that the Z sex chromosome, by itself, is not the sole basis of the difference in reciprocal cross mortality; otherwise, the pure lines should differ as much in mortality as the reciprocal crosses. In this study, mortality of the pure lines was lower than that of the reciprocal crosses. This leads to the tentative hypothesis that the genetic basis of the difference in laying house mortality between Leghorn x heavy reciprocal crosses is due to interactions involving autosomes and maternal effects. Yet, the role of the sex chromosome and sex hormones cannot be ruled out of the genetic basis of disease resistance.

In searching for genetic mechanisms of disease resistance, it seems logical to look for genetic resistance to specific diseases (Hutt, 1958). Over the 2 year period, 77.4 percent of the posted GxH pullets died of leukosis compared with 59.0 percent in the HxG reciprocal cross pullets. The number of pullets dying from other causes were few. Thus, leukosis would automatically account for most of the difference in reciprocal cross mortality. Presumably most of this would be lymphoid leukosis because the flocks were vaccinated for Marek's disease in 1972. Furthermore, the incidence of Marek's disease was usually low within the test period between the ages of 20 and 63 weeks.

Crittenden et al. (1972) concluded that genetic resistance to lymphoid leukosis is highly complex. The autosomal resistance to leukosis/sarcoma viruses, designated a "first line of defense", was shown to be under the control of at least 3 genetic loci. Other factors such as the immune response to the virus were found to form a second line of defense against lymphoid leukosis. A significant maternal effect on lymphoid leukosis mortality was also reported. In a previous study, Kawahara (1960) reported that resistance and susceptibility to visceral leukosis were largely controlled by maternal factors.

The transmission of infection through the hatching egg is an example of a maternal effect. Burmester and Waters (1955) concluded that chickens exposed to visceral lymphomatosis (VL), transfer the VL viruses to their offspring through the egg. Even though the infection may not kill the maternally infected chicks, the latter may infect chicks from uninfected parents during the brooding period. This report lends little support to the possibility that congenital infection of the heavy line in the present study was responsible for the higher lymphoid leukosis of the GxH cross.

Resistance to infection is also affected by maternal antibodies transferred from dam to offspring via the egg. Burmester et al. (1957) showed that offspring of chickens,

treated with VL vaccine, were more resistant to the disease than offspring of untreated dams. Higher mortality of the reciprocal crosses than that of the pure lines, rules out any protective role of antibodies transferred from parents to offspring. The heavy line maternal effect on lymphoid leukosis mortality found in this study, seems not to be associated with a transfer of pathogens or antibodies via the hatching egg.

As to autosomes, an evaluation of the tests on cellular resistance to the A and B avian leukosis viruses suggests that the Leghorn line GL probably has a higher frequency of genes for cellular susceptibility to lymphoid leukosis than the heavy line. This fact may explain the effect of Leghorn autosomes in increasing lymphoid leukosis mortality.

The small and nonsignificant difference in mortality found between G^nH and H^nG pullets, shows that the Z chromosome, alone, does not influence lymphoid leukosis. The genetic mechanism of resistance to lymphoid leukosis, seemingly, has a complex basis involving genes for cellular susceptibility and a heavy line maternal effect. The maternal effect might be some form of extra-nuclear inheritance (Hutt, 1964).

Except for leukosis, the number of dead pullets was low in the other diagnostic classes to speculate on the role of genetic factors. None could account for the large difference in total mortality between GxH and HxG crosses.

B Blood Group Effects

The purpose of the second part of the present study was an attempt to show more specifically the effects of the B locus on mechanisms of disease resistance. There seems to be little question that the B blood group locus is associated with economic traits in chickens. That the B locus influences fertility, hatchability, body weight, egg production, and livability has been reported by Gilmour (1960), Briles (1960), as well as others. More recently, Nordskog et al. (1973) reported lower egg production and higher adult mortality of the B^1B^1 homozygote.

In this study, mortality of the B^1 homozygous pullets was significantly higher than that of the B^1 heterozygous controls. There seemed to be a general trend of increased viability of the B^1B^1 blood type group pullets over successive generations (Nordskog et al., 1973). The present results reported here seem to fall in line with this general trend.

The higher mortality in the B^1B^1 pullets seems not to be accounted for by any single disease. In the S1 line, mortality of the posted birds was diagnosed mainly as leukosis or from certain unknown causes where the most common symptom was diarrhea. The differences in mortality from leukosis, or incidence of diarrhea between the B^1 homozygotes and heterozygotes, was not statistically significant. From similar autopsy studies in the S1 line, carried out in 1968 and 1969,

the B^1 homozygotes were consistently higher in mortality than the heterozygotes. Leukosis accounted for about 53 percent of the deaths of the former and nearly 70 percent of the latter group. Thus, the 4 years of autopsy data failed to reveal any specific disease responsible for higher mortality of B^1B^1 pullets. A different way to attack the problem, therefore, was considered. In 1972 a special immunological study was initiated. The approach was to look for possible differences in antibody response to a variety of antigens, including cellular antigen, 2 soluble antigens and a viral antigen. The most notable result was the marked difference between genotypes in titer to inoculation with S. pullorum. The B^1B^1 genotype is evidently deficient in producing antibodies as indicated by the consistent difference found in each of the 2 years, 1972 and 1973.

The B^1B^1 pullets were also poorer producers of antibodies to the soluble antigen, ferritin, although the effect was not statistically significant. Yet, the average titer in the B^1B^1 group was only 73 percent as that of the B^1B^2 control. These results suggest that the B locus may also control the antibody production against soluble antigens.

The genetic control of the immune response may have either a polygenic or single gene basis. Adams and Sobey (1961) and Sobey et al. (1966) showed that the inheritance of certain specific antibody responses to antigens in rabbits

and mice is quantitative.

In the present study, no difference between genotypes could be detected in the immune response from inoculating the soluble antigens, bovine serum, albumin, and parainfluenza 3. This suggests that the genetic regulation of the immune response, in which the B blood group locus is involved, is more specific than general. Sobey et al. (1966) demonstrated that rabbits, which responded normally to certain antigens, failed to produce antibodies to bovine plasma albumin.

The B locus not only exerts some control over antibody response, but is also known to be the major histocomatibility locus in chickens (Schierman and Nordskog, 1964). Thus, it seems to play a role in both humoral and cellular immunity. This would imply that the B gene is involved in the regulation of both the thymus- and bursa-dependent immune systems.

The question whether the poor viability of B^1B^1 genotype is a pleiotropic effect of the B^1 gene or a direct effect of a fitness gene linked to it, remains unanswered (Nordskog et al., 1973). Likewise, the effect of the B^1 gene on the immune response may be either a pleiotropic or a linked gene effect.

An immune response region Ir-1 is known to be associated with the H-2 locus the major histocompatibility locus in mice. Likewise, in guinea pigs an Ir gene, PLL, is linked

to the major histocompatibility locus of that species. Since the B locus is the major histocompatibility locus in chickens, the results of this study are highly suggestive that an Ir region exists within or is closely linked to the B locus.

SUMMARY

Genetic mechanisms of resistance to diseases in chickens were investigated first with respect to the quantitative effect of sex chromosomes and secondly with respect to the influence of the B blood group locus on disease resistance.

For the sex chromosome study, line GL from the Leghorn breed, line H developed from a heavy breed cross foundation and their reciprocal crosses GxH and HxG were used. In addition, 2 synthetic lines G^nH and H^nG, segregating for Z chromosome were used. The G^nH and H^nG males were crossed every generation with the F_1 females GxH and HxG, respectively. This was done in order to introduce the Z_1 chromosome of GxH into G^nH and the Z_2 chromosome of HxG into the H^nG line. Progeny were produced each year from pen matings of 6 males with 80-90 females for each line.

Total laying house mortality was recorded on a flock of 934 pullets in 1971 and a flock of 996 pullets and 296 cockerels in 1972. After autopsying all dead birds, they were placed into one of the following classes: leukosis, fatty liver, diarrhea, enteritis and peritonitis, cannibalism, and reproductive disorders. The experimental design used for this study made it possible to estimate a maternal line effect (m), an autosomal effect (a), and a heterosis effect (h) in addition to the primary sex chromosome difference

between lines (z).

The GxH cross had significantly higher total laying house mortality than its reciprocal (HxG), i.e., 40.9 versus 27.3 percent. No z effect on total mortality could be detected. The a, m, and h estimates were -6.7, 15.5, and 15.8 percent, respectively, indicating that the heavy line maternal effect and the GL line autosomes contributed to total mortality.

Most of the autopsied chickens were diagnosed as leukosis and the specific disease was assumed to be lymphoid leukosis. Mortality from leukosis seemed to be associated with a heavy line maternal effect and with Leghorn autosomes. The maternal effect may represent a difference in extra-nuclear genetic factors transferred via the hatching eggs to the cross with eggs from the H line showing less immunity. The Leghorn line was found to have higher frequency of genes for cellular susceptibility to leukosis/sarcoma viruses. This may explain the adverse effect of Leghorn autosomes on livability. The high estimate for heterosis was obtained due to high mortality of crossbred, compared with the parental pure lines.

For the second part of the study, pullets of 3 different blood group genotypes, B^1B^1, B^1B^2, and B^1B^{19} in a Leghorn line S1 were compared in adult mortality and in their ability to produce antibodies. Mortality data were collected on 451 pullets in 1971 and 1972. The same autopsy procedures were

used as in the sex chromosome study. Pullets of the B^1B^1 genotype had significantly higher mortality than the B^1 heterozygotes. Leukosis was the main cause of death although diarrhea was the sole diagnostic finding in 42.4 percent of posted birds. The differences between B genotypes in leukosis or diarrhea mortality were not statistically significant.

In a special experiment, conducted in 1972 and 1973, the B^1B^1 proved to be a poor producer of antibodies to Salmonella pullorum compared with pullets of the B^1 heterozygote genotypes. The difference between the genotypes was highly significant in both years of the experiment. Another experiment also showed that the B^1B^1 produced lower titers of antibodies to iron binding protein, ferritin, than the B^1B^2, although the difference was not statistically significant. Tests between genotypes in the immune response to injections of bovine serum albumin and to parainfluenza 3 showed non-significant differences in titers. The subnormal ability of the B^1B^1 genotype to produce antibodies to certain antigens may account for the higher mortality of this genotype.

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APPENDIX

Table 19a. Antibody titers to Salmonella pullorum (1972)

Days post inoculation	Genotypes B^1B^1 7	B^1B^1 14	B^1B^1 21	B^1B^2 7	B^1B^2 14	B^1B^2 21	B^1B^{19} 7	B^1B^{19} 14	B^1B^{19} 21
	640	320	80	640	80	0	640	320	40
	160	160	80	1280	160	80	320	160	80
	80	20	0	40	0	0	320	80	40
	40	0	0	320	160	80	640	80	0
	40	0	0	320	80	40	320	40	0
	40	20	20	1280	80	40	160	0	0
	40	20	20	160	40	0	640	80	-
	160	80	20	640	80	20	640	80	40
	20	20	0	640	40	20	160	20	0
	80	40	0	2560	160	80	640	160	80
	40	20	0	1280	320	80	640	160	80
	80	40	0	320	80	40	320	40	20
	160	40	20	320	40	0	320	40	0
	80	20	0	640	160	40	640	80	40
	80	0	0	1280	80	40	320	40	40
	640	80	40	160	40	0	80	0	0
	320	80	40	320	40	0	320	160	80
	20	20	0	640	40	0	320	40	40
	80	20	0	160	40	0	320	40	0
	640	160	80	640	80	80	320	80	80
Mean	172	58	20	682	90	32	404	85	35
Mean $\log_{10}$	1.9783	1.3818	0.7058	2.6027	1.7928	1.0064	2.5502	1.6826	1.0752
σ_x	0.4670	0.6907	0.8208	0.4244	0.4992	0.8605	0.2445	0.6448	0.8574
$\sigma_{\bar{x}}$	0.1044	0.1542	0.1833	0.0948	0.1113	0.1923	0.0538	0.1438	0.1967

Table 19b. Antibody titers to Salmonella pullorum (1973)

Days post incoculation	Genotypes B^1B^1 4	7	10	14	B^1B^2 4	7	10	14
	0	0	40	0	20	40	40	40
	0	320	160	80	80	160	320	160
	0	40	80	20	0	160	80	80
	0	320	320	320	40	640	320	80
	0	320	20	20	80	320	320	80
	40	320	160	40	20	640	640	80
	20	320	80	40	40	160	320	80
	0	160	80	20	20	640	320	80
	0	160	80	20	40	320	320	160
	0	80	80	40	40	80	160	20
	40	320	160	160	80	320	320	80
	0	80	80	20	20	320	160	40
	20	20	20	20	40	320	80	40
	20	160	80	40	40	640	320	160
	0	80	160	20	0	160	160	20
Mean	9	180	106	57	37	328	258	80
Mean $\log_{10}$	0.4738	2.0170	1.9231	1.4751	1.3685	2.4047	2.3245	1.8228
σ	0.6991	0.6721	0.3309	0.5494	0.5944	0.3535	0.3176	0.2891
$\sigma_{\bar{x}}$	0.1802	0.1734	0.0854	0.1417	0.1532	0.0911	0.0818	0.0741

Table 20. Antibody titers to bovine serum albumin (1972)

Days post inoculation	Genotypes B^1B^1			B^1B^2			B^1B^{19}		
	7	14	21	7	14	21	7	14	21
	20	80	80	320	320	160	80	80	-
	20	40	80	40	320	160	160	160	40
	80	80	80	80	80	80	640	80	20
	10	0	10	160	160	160	320	80	-
	320	80	80	80	160	40	80	640	160
	10	20	20	80	40	80	320	80	40
	40	80	80	40	160	160	80	320	-
	40	160	40	40	40	20	20	20	40
	640	40	40	640	640	160	40	40	80
	80	640	160	160	320	320	640	80	160
	20	80	80	160	640	640	320	40	80
	40	640	80	320	320	320	40	80	80
	320	40	20	40	80	80	80	80	80
	640	640	320	80	80	80	160	640	640
	320	640	80	640	160	160	80	40	40
	320	80	20	40	40	40	160	640	320
	160	40	10	20	20	20	-	-	-
	40	640	160	160	40	40	20	160	40
	80	40	80	640	640	640	40	40	80
	320	80	160	80	20	20	40	80	40
Mean	176	207	84	191	214	169	174	177	121
Mean $\log_{10}$	1.9181	1.9437	1.7676	2.0536	2.0987	2.0084	2.0139	2.0140	1.8842
σ_x	0.5897	0.6716	0.4081	0.4527	0.4909	0.4608	0.4620	0.4396	0.3879
$\sigma_{\bar{x}}$	0.1315	0.1500	0.0911	0.1009	0.1095	0.1029	0.1058	0.1004	0.0969

Table 21. Antibody titers to ferritin (1973)

Days post inoculation	Genotypes B^1B^1 4	7	10	14	B^1B^2 4	7	10	14
	80	1280	1280	160	640	5120	5120	2560
	80	640	2560	640	2560	10240	2560	640
	40	640	640	80	1280	640	640	320
	2560	10240	5120	1280	1280	1280	1280	640
	2560	1280	1280	320	320	1280	-	320
	1280	2560	2560	320	1280	2560	2560	320
	2560	5120	5120	640	1280	1280	2560	640
	1280	5120	1280	640	640	10240	1280	320
	2560	2560	1280	320	5120	5120	1280	1280
	640	2560	5120	320	5120	10240	5120	1280
	1280	20480	5120	1280	1280	20480	20480	2560
	2560	5120	2560	640	1280	1280	1280	320
	5120	5120	5120	1280	10240	-	10240	2560
	5120	-	10240	2560	5120	5120	2560	640
	1280	2560	-	640	2560	20480	20480	2560
Mean	1933	4662	3520	741	2667	6811	5531	1130
Mean $\log_{10}$	3.0068	3.4727	3.4297	2.7258	3.2476	3.6017	3.4942	2.9063
σ_x	0.6698	0.4286	0.3433	0.3850	0.4079	0.4453	0.4637	0.3714
$\sigma_{\bar{x}}$	0.1729	0.1144	0.0916	0.0984	0.1048	0.1322	0.1236	0.0959

Table 22. Antibody titers (HI) to parainfluenza 3 virus (1973)

Days post inoculation	Genotypes B^1B^1				B^1B^2			
	7	10	14	21	7	10	14	21
	0	0	60	120	0	0	60	60
	0	480	60	60	60	60	60	60
	0	960	0	0	60	60	-	60
	30	480	0	0	60	60	60	60
	60	480	60	0	0	0	0	0
	30	480	0	0	0	0	0	0
	30	480	30	30	60	60	60	60
	30	60	60	60	30	30	30	60
	60	60	0	0	60	30	-	0
	60	0	60	30	60	60	60	60
	0	60	30	30	30	480	30	0
	60	30	0	0	30	60	60	120
	30	0	60	60	30	60	60	60
	-	120	120	120	60	60	60	120
	60	30	-	30	60	60	60	60
Mean	32	248	38	36	40	72	46	52
Mean $\log_{10}$	1.1626	1.7837	1.1216	1.0267	1.3422	1.4426	1.4583	1.3441
σ_x	0.7743	1.0448	0.8790	0.8882	0.7069	0.7924	0.6565	0.8452
$\sigma_{\bar{x}}$	0.2068	0.2696	0.2347	0.2291	0.1824	0.2044	0.1819	0.2181