SELECTIVE ADVANTAGE OF HETEROZYGOTES AT B BLOOD GROUP LOCUS IN THE CHICKEN

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A multiple allelic system at a locus such as a B blood group system of the chicken can give rise to genotypes with different viabilities. Under certain conditions, the genes involving such a system can exist in a state of equilibrium, being known as a balanced polymorphism. In the general case, a necessary condition for stable polymorphism is that the viability of each homozygote be less than the mean viability in the population. Roughly speaking, the heterozygotes can be more viable than any of the homozygotes (Mandel 1959).

Two or more alleles of B blood group system were demonstrated to exist in several closed populations of chickens (Briles *et al.* 1957; Gilmour 1959; Fujio and Kondo 1970). This type of evidence argues powerfully for heterozygous advantage. Fujio and Kondo (1970) revealed apparent selective advantage of heterozygotes, that is, the observed frequency of heterozygote in each population was higher than the expected one. Several workers have shown evidence that heterozygotes of blood group loci were superior in term of fertility, hatchability, viability, and egg production (Briles and Krueger 1955; Briles and Allen 1961; Allen 1962; Allen and Gilmour 1962; Morton *et al.* 1965).

The superiority of heterozygotes in regard to each character such as fertility, hatchability, and viability should be proved by the cross experiment. The present paper is to point out an abnormal segregation of B blood group alleles, and to demonstrate the superiority of heterozygotes in hatchability, an important component of fitness.

MATERIALS AND METHODS

Four inbred strains and one breed were used for the present work. These four inbred strains were BM-C, NG-B, WL-M, and CON. The birds of these strains were homozygous for the genes determining blood group antigens as described previously (Fujio *et al.* 1970). In addition, one breed was Rhode Island Red (RIR). Its genotype concerned with the blood group gene B was determined by the cross test. The genotype of B blood group system and plumage color in four inbred strains, one breed, and their F₁ hybrids are summarized in Table 1.

The serological procedures and the nomenclature used in the present work had been previously reported (Fujio *et al.* 1970). All blood samples were collected from wing vein

Table 1. The genotype of B blood group system and of other character in four inbred strains, one breed, and their F₁ hybrids

	Origin	Genotype of	
		B blood group	Plumage color
<i>Strain</i>			
BM-C	Black Minorca C	B^A/B^A	$i/i; E/E$
NG-B	Nagoya B	B^B/B^B	$i/i; e/e$
RIR	Rhode Island Red	B^G/B^G	$i/i; e^v/e^v$
CON	Cross between White Cornish and New Hampshire	B^K/B^K	$i/i; e/e$
WL-M	White Leghorn M	B^M/B^M	I/I
<i>F₁ hybrid</i>			
NG-B×BM-C		B^A/B^B	$i/i; E/e$
RIR×BM-C		B^A/B^G	$i/i; E/e^v$
BM-C×CON		B^A/B^K	$i/i; E/e$
BM-C×WL-M		B^A/B^M	I/i
NG-B×CON		B^B/B^K	$i/i; e/e$

of 3 weeks old chicks. Detection of antigens was performed with five iso-immune antisera involved in the B blood group system (named A, B, G, K, and M). For hemagglutination tests, the titer of antisera was adjusted with saline to 1:4, and the twofold serial dilutions of each antiserum were prepared in a plastic agglutination plate. Equal volume of 2 percent red cell suspension (V/V) was added to 0.1 ml. of each diluted antiserum. Hemagglutination patterns were recorded at 30-60 minutes after mixing.

RESULTS

Table 2 shows F₂ segregation of the B blood group alleles. Their segregation ratios do not agree with Mendelian segregation ratio, that is, occurrence of heterozygous genotypes is higher than that expected on the basis of Mendelian segregation in all cases. The differences between the observed numbers and expected ones were statistically significant in all cases except one.

The F₂ segregations of the other character genes, namely the black-red melanin distribution (E, e , and e^v) and dominant white (I) in the color of plumage were simultaneously examined as control in the crosses for the B blood group alleles (Table 3). Their segregation ratios were highly accordant with Mendelian segregation ratio ($P > 0.80$). Table 3 also shows the F₂ segregation of the other character genes such as those governing diluting black plumage (Bl), red cell agglutinin in response to tissue hemagglutinin (Th), and creeper (Cp) which were examined in the course of other experiments at our laboratory. It indicated that their segregation ratios corresponded to Mendelian segregation ratio ($P > 0.50$). Thus it is recognized that the F₂ segregation of only B blood group alleles is abnormal.

Table 2. F_2 segregation of antigen in B blood group locus
 F_2 (NG-B $\times$ BM-C)

	B^A/B^A	B^B/B^B	B^A/B^B	Total	χ^2
Observed	35	50	115	200	6.750
Expected	50.0	50.0	100.0	200.0	

F_2 (RIR $\times$ BM-C)

	B^A/B^A	B^G/B^G	B^A/B^G	Total	χ^2
Observed	26	19	61	106	3.339
Expected	26.5	26.5	53.0	106.0	

F_2 (BM-C $\times$ CON)

	B^A/B^A	B^K/B^K	B^A/B^K	Total	χ^2
Observed	50	30	122	202	12.692
Expected	50.5	50.5	101.0	202.0	

F_2 (BM-C $\times$ WL-M)

	B^A/B^A	B^M/B^M	B^A/B^M	Total	χ^2
Observed	25	18	83	126	13.476
Expected	31.5	31.5	63.0	126.0	

F_2 (NG-B $\times$ CON)

	B^B/B^B	B^K/B^K	B^B/B^K	Total	χ^2
Observed	24	30	136	190	39.389
Expected	47.5	47.5	95.0	190.0	

The reciprocal backcross tests were performed as shown in Table 4. In the crosses between sire B^B/B^B and dam B^B/B^K and between sire B^B/B^K and dam B^B/B^B , a highly significant deviation from the Mendelian proportion (1:1) was observed, that is, occurrence of heterozygous genotypes was higher than that of homozygous genotypes. Similar result was obtained in the crosses between sire B^K/B^K and dam B^B/B^K , and between sire B^B/B^K and dam B^K/B^K . Thus, it suggested that an abnormal segregation was not due to a maternal effect.

If the abnormal segregation is due to a difference in embryonic mortality associated with the genotypes, the hatchability should be varied for the genotypes. Fertilized eggs of each genotype were calculated by assuming that the segregation of fertilized eggs in F_2 and backcross had corresponded to Mendelian ratio, and the hatchability of each genotype was estimated. The observed numbers at 3 weeks old were representative of the numbers at hatching, since the survival rate of the newly hatched chicks to 3 weeks old was almost 100 percent. Tables 5 and 6 represent a significant difference in hatch-

Table 3. F₂ segregation of several characters
Plumage color in the crosses for the B blood group alleles
F₂ (NG-B×BM-C)

	<i>E/E</i>	<i>e/e</i>	<i>E/e</i>	Total	χ^2
Observed	63	65	118	246	0.438
Expected	61.5	61.5	123.0	246.0	

F₂ (RIR×BM-C)

	<i>E/E</i>	<i>e^v/e^v</i>	<i>E/e^v</i>	Total	χ^2
Observed	26	26	58	110	0.327
Expected	27.5	27.5	55.0	110.0	

F₂ (BM-C×WL-M)

	<i>I/I</i>	<i>i/i</i>	<i>I/i</i>	Total	χ^2
Observed	41	37	73	151	0.363
Expected	37.75	37.75	75.50	151.0	

Diluting black plumage

	<i>Bl/Bl</i>	<i>bl/bl</i>	<i>Bl/bl</i>	Total	χ^2
Observed	52	48	96	196	0.244
Expected	49.0	49.0	98.0	196.0	

Th agglutinin in response to tissue hemagglutinin

	<i>Th/Th</i>	<i>th/th</i>	<i>Th/th</i>	Total	χ^2
Observed	36	30	71	137	0.795
Expected	34.15	34.15	68.30	137.0	

Creeper

	<i>Cp/Cp</i>	<i>cp/cp</i>	<i>Cp/cp</i>	Total	χ^2
Observed	55	48	110	213	0.690
Expected	53.25	53.25	106.5	213.0	

ability as compared homozygotes with heterozygotes. Heterozygotes showed higher hatchability than homozygotes. Furthermore it was observed that hatchability of heterozygotes was same as that of F₁ hybrids in each combination of the alleles.

The hatchability of each homozygote was lower than the mean hatchability in F₂ and backcross. Homozygote showed varied hatchability from one cross to another. For example, the hatchability of *B^A/B^A* were 53.8, 59.9, 65.0, and 76.3 percent in F₂ segregation

Table 4. Segregation of antigen in B blood group locus in the backcrosses

NG-B (♂) $\times$ F₁ (NG-B $\times$ CON) (♀)

	B^B/B^B	B^B/B^K	Total	χ^2
Observed	25	62	87	15.747
Expected	43.5	43.5	87.0	

F₁ (NG-B $\times$ CON) (♂) $\times$ NG-B (♀)

	B^B/B^B	B^B/B^K	Total	χ^2
Observed	27	63	90	14.400
Expected	45.0	45.0	90.0	

CON (♂) $\times$ F₁ (NG-B $\times$ CON) (♀)

	B^K/B^K	B^B/B^K	Total	χ^2
Observed	18	57	75	20.280
Expected	37.5	37.5	75.0	

F₁ (NG-B $\times$ CON) (♂) $\times$ CON (♀)

	B^K/B^K	B^B/B^K	Total	χ^2
Observed	22	62	84	19.047
Expected	42.0	42.0	84.0	

of NG-B $\times$ BM-C, BM-C $\times$ WL-M, RIR $\times$ BM-C, and BM-C $\times$ CON, respectively. The variation in hatchability of homozygote could be attributed to the partner. The genetic make-up of partner may have influence on progeny performance.

Although quantitative analysis on embryonic mortality was not carried out through the incubation period, it was observed that the main contributions to embryonic mortality had come from the high mortality periods of 16-21 days of incubation. Thus, the difference in embryonic mortality among genotypes of the B blood group locus is responsible for the cause of an abnormal segregation.

DISCUSSION

The deviation of genotypic class numbers from those expected was found in F₂ and backcross segregation of only B blood group system due to the fact that the number of heterozygous genotype is larger than that expected. The supposition may not always be justified that segregation of B blood group genotypic classes is accordant with Mendelian ratio. The existence of this disturbance in the ratio underlines the need to draw attention on itself, when blood grouping tests are carried out for the purpose of progeny test, genetic analysis and so on.

Table 5. Effects of the genotypes of B blood group system on hatchability
NG-B×BM-C

Genotype	B^A/B^A	B^B/B^B	B^A/B^B	Total or mean
No. of hatch	35	50	115	200
No. of fertile eggs	60	60	120	240
Hatchability of F_2	53.8%	83.3%	95.3%	83.3%
Hatchability of F_1			96.7%	96.7%

RIR×BM-C

Genotype	B^A/B^A	B^G/B^G	B^A/B^G	Total or mean
No. of hatch	26	19	61	106
No. of fertile eggs	40	40	80	160
Hatchability of F_2	65.0%	47.6%	76.3%	66.3%
Hatchability of F_1			78.6%	78.6%

BM-C×CON

Genotype	B^A/B^A	B^K/B^K	B^A/B^K	Total or mean
No. of hatch	50	30	122	202
No. of fertile eggs	65.5	65.5	131.0	262
Hatchability of F_2	76.3%	45.8%	93.1%	77.1%
Hatchability of F_1			96.3%	96.3%

BM-C×WL-M

Genotype	B^A/B^A	B^M/B^M	B^A/B^M	Total or mean
No. of hatch	25	18	83	126
No. of fertile eggs	41.75	41.75	83.5	167
Hatchability of F_2	59.9%	43.1%	99.4%	75.4%
Hatchability of F_1			96.1%	96.1%

NG-B×CON

Genotype	B^B/B^B	B^K/B^K	B^B/B^K	Total or mean
No. of hatch	24	30	136	190
No. of fertile eggs	70.25	70.25	140.5	281
Hatchability of F_2	34.2%	42.7%	96.8%	67.6%
Hatchability of F_1			96.3%	96.3%

The mechanism operating to cause the observed disturbance in the segregation ratio should propose two suppositions. The first suggests an abnormal segregation of oocytes in the dam during meiosis. Perramon and Merat (1968) reported that a highly significant heterogeneity in segregation ratios between heterozygous dams at blood group loci W^6/W^0 and R^4/R^0 was observed in crosses sire W^0/W^0 ×dam W^6/W^0 of Wyandotte strain

Table 6. Effects of the genotypes of B blood group system on hatchability

NG-B (σ) $\times$ F₁ (NG-B $\times$ CON) (φ)

Genotypes	B^B/B^B	B^B/B^K	Total or mean
No. of hatch	25	62	87
No. of fertile eggs	63	63	126
Hatchability	39.7%	98.4%	69.0%

F₁ (NG-B $\times$ CON) (σ) $\times$ NG-B (φ)

Genotypes	B^B/B^B	B^B/B^K	Total or mean
No. of hatch	27	63	90
No. of fertile eggs	66.5	66.5	133
Hatchability	40.6%	94.7%	67.7%

CON (σ) $\times$ F₁ (NG-B $\times$ CON) (φ)

Genotypes	B^K/B^K	B^B/B^K	Total or mean
No. of hatch	18	57	75
No. of fertile eggs	60	60	120
Hatchability	30.0%	95.0%	62.5%

F₁ (NG-B $\times$ CON) (σ) $\times$ CON (φ)

Genotypes	B^K/B^K	B^B/B^K	Total or mean
No. of hatch	22	62	84
No. of fertile eggs	62.5	62.5	125
Hatchability	35.2%	99.2%	67.2%

and sire $R^0/R^0 \times$ dam R^4/R^0 of Rhode Island strain respectively. The reciprocal cross test in the present work suggested that an abnormal segregation was not due to maternal effect. However, a possibility that there is an abnormal segregation of oocytes in the dam may remain to be investigated, since small numbers of dams were used for the cross experiment in this work.

In the second supposition, a difference in embryonic mortality associated with the genotypes should be considered. Earlier works on the effects of blood groups on hatchability concerned the specialized situations of inbred lines or intercrossbreds (review by Gilmour 1960; Allen 1962; Allen and Gilmour 1962). They had analyzed the variance in hatchability associated with parental genotype. Recently, Morton *et al.* (1965) showed that the B blood group genotypes of zygotes but not of dams were associated with marked differences in mortality over the whole incubation periods.

The present data showed that the B blood group locus was generally overdominant in its effects on hatchability. Heterozygotes are superior in hatchability to homozygotes, and the hatchability of each homozygote is lower than the mean hatchability in F₂ and

backcross. This fulfils the simplest of Mandel's (1959) necessary conditions for a stable polymorphism, that is, the viability of each homozygote should be less than the mean population viability in a state of equilibrium.

The estimated time of embryonic death is reasonable from the time of appearance of antigen at the B blood group system. Fujio *et al.* (1970) reported that several antigens of the B blood group system appeared at 7-12 days of incubation. It was also found by Morton *et al.* (1965) that the main contribution to the overall embryonic mortality came from the high mortality period from 16 to 19 days of incubation.

Thus, the evidence was obtained that the B blood group genotypes of zygotes were associated with powerful selective effects through hatchability. It is not however sufficient in itself to explain the maintenance of the stable polymorphism, since it is necessary at a next step to take into account selective effects in the other periods of the life cycle though hatchability is an important one.

SUMMARY

In cross experiment, the deviation of genotypic class numbers from those expected was found in F_2 and backcross segregation of only B blood group system, due to the fact that the number of heterozygous genotypes was larger than that expected.

The B blood group genotypes of zygotes associated themselves with marked differences in embryonic mortality over the whole incubation periods, especially at periods of 16-21 days of incubation.

The hatchability of each homozygote was lower than the mean hatchability in F_2 and backcross. The maintenance of stable polymorphism could be explained by differences in mortality among genotypes.

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