Mode of Inheritance of Unselected Traits in Lines of Chickens Selected for High or Low Antibody Response to Sheep Red Blood Cells. 2. Heterophils, Lymphocytes, and Hematocrits

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ABSTRACT The nuclear lines for this experiment were White Leghorns that had undergone long-term selection for high (HH) or low (LL) antibody response to sheep red blood cell antigen(s). Sixteen progeny types consisting of parental lines, reciprocal F_1 and F_2 crosses, and backcrosses were produced in a single hatch from age-contemporary parents. At 30 d of age, blood was obtained from a random sample of 10 males per progeny type (n = 160) and slides prepared for subsequent determination of number of heterophils and lymphocytes. Twelve days later, blood was collected from random samples of 10 males and 10 females per progeny type (n = 320) for measuring hematocrits. There were no differences between parental lines for heterophils, lymphocytes, or the heterophil:lymphocyte ratio. Reciprocal effects were evident in the F_1 crosses and directional heterosis was present in one cross but not the other. Neither maternal heterosis nor recombination effects were significant for either heterophils or lymphocytes. Although hematocrits were similar for males and females and parental lines, sex-linked and recombination effects appeared to be important.

(*Key words*: chicken, sheep red blood cells, hematocrits, heterophils, lymphocytes)

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INTRODUCTION

An animal's protection from disease is based, in part, on phagocytic, cell-mediated, and humoral immunity. In birds, the heterophils are phagocytic cells whose main role is protection against invading microorganisms (Powell, 1987), whereas primary functions of lymphocytes involve cell-mediated and humoral immunity. Heterophils increase and lymphocytes decrease when chickens are stressed, so that the ratio between them is a good index of response to a stressor (Gross and Siegel, 1983; Siegel, 1995). There is a genetic component to heterophil and lymphocyte responses to stressors (Gross and Siegel, 1985) and their ratio has been used as a heritable selection criterion for heat resistance in chickens (Al-Murrani *et al.*, 1997). Gene action models for heterophils and lymphocytes in unstressed chickens, however, have not been investigated.

Relative hematocrit values (packed cell volume) are quantitatively inherited, with heritabilities of 0.39 and 0.27 from paternal and maternal half-sib correlations, respectively (Washburn, 1967). Although Shlosberg *et al.* (1996) demonstrated in chickens that hematocrit values could be changed through selection, information on gene action models for this trait is lacking. This paper reports on the modes of inheritance of heterophils, lymphocytes, and hematocrits as measured in nuclear lines selected for high and low antibody response to SRBC, their F_1, F_2, and backcross populations.

MATERIALS AND METHODS

This experiment involved two lines of White Leghorn chickens derived from the same base population but selected divergently for high (HH) or low (LL) antibody response 5 d after a single intravenous injection of 0.1 mL of 0.25% suspension of SRBC antigen(s) (Siegel and Gross, 1980; Martin *et al.*, 1990). The methods of mating and husbandry for the chicks used in this experiment were described by Boa-Amponsem *et al.* (1998). Briefly, matings were made between age-contemporary chickens from the S_{22} generation of these lines to produce the parental lines and reciprocal F_1 crosses. These four populations were then mated to produce 16 progeny types consisting of parental, reciprocal F_1, F_2, and backcrosses. At hatch, 100 straight-run chicks of each progeny type were wing-banded, vaccinated for Marek's disease, and placed in floor pens with wood shavings as

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Abbreviation Key: HH = high antibody response line; LL = low antibody response line.

TABLE 1. Mean number of heterophils, lymphocytes, and heterophil:lymphocyte ratios at 30 d of age for cockerels from all mating combinations of lines HH[1] and LL[1]

Progeny[2]	Heterophils[3]	Lymphocytes[3]	Heterophil:lymphocyte ratio[3]
Parental lines and F_1			
HHHH	20.7	39.3	0.56
HHLL	14.4	45.6	0.33
LLHH	19.9	40.1	0.51
LLLL	17.7	42.3	0.45
Backcross to HH parental line			
HHHL	19.8	40.2	0.53
HHLH	18.6	41.4	0.47
HLHH	18.5	41.5	0.47
LHHH	18.3	41.7	0.47
Backcross to LL parental line			
LLHL	20.2	39.8	0.52
LLLH	17.5	42.5	0.45
HLLL	18.4	41.6	0.45
LHLL	17.3	42.7	0.42
F_2			
HLHL	20.2	41.6	0.47
HLLH	17.5	41.1	0.47
LHHL	18.4	45.6	0.34
LHLH	17.3	43.0	0.41
Pooled SEM	0.4	0.4	0.2

[1]HH and LL were selected for high and low antibody response to SRBC, respectively.

[2]The first two letters designate the sire and the second two letters the dam population for the mating combination.

[3]Genetic contrasts are shown in Table 2.

litter. Chicks consumed *ad libitum* a mash diet containing 20% CP and 2,685 kcal ME/kg. Lighting and water were available continuously. These husbandry procedures were consistent with those under which the lines were selected.

At 30 d of age, blood was obtained from the brachial vein of 10 males from each mating combination and mixed with ETDA as the anticoagulant. Slides were prepared for determining the number of heterophils and lymphocytes as described by Gross and Siegel (1983). All slides were coded and a total of 60 cells were classified as heterophils or lymphocytes by the same individual. At 42 d of age, blood was collected from the brachial vein in heparinized microhematocrit tubes from 10 females and 10 males from each mating combination. Duplicate samples were centrifuged and the percentage packed cell volume was recorded as the hematocrit.

Hematocrits and heterophil:lymphocyte ratios were transformed to arc sine square roots prior to analysis. Genetic analysis for heterophils, lymphocytes, and heterophil:lymphocyte ratios were conducted for males only. Genetic analyses for hematocrits were conducted within sexes and with sexes pooled in order to obtain information on sex-linked and maternal effects. The models used for comparing paternal lines, reciprocal effects, heterosis, maternal heterosis, and recombination effects were the same as those described by Boa-Amponsem *et al.* (1998) with specific comparisons also made. Calculations for scaling tests A, B, and C for an additive-dominance model (Mather and Jinks, 1982) were consistent with those of Boa-Amponsem *et al.* (1998). The formulae were:

$$A = 2\overline{BC}_{HH} - HH - [(HL + LH)/2],$$
$$B = 2\overline{BC}_{LL} - LL - [(HL + LH)/2],$$

and

$$C = 4\overline{F}_2 - 2\overline{F}_1 - HH - LL$$

where,

$$\overline{BC}_{HH} = (HHHL + HHLH + HLHH + LHHH)/4$$

and

$$\overline{BC}_{LL} = (LLHL + LLLH + HLLL + LHLL)/4.$$

RESULTS

Heterophils, Lymphocytes and the Ratio

Mean heterophils, lymphocytes, and heterophil:lymphocyte ratios for males at 30 d of age are presented in Table 1 with genetic contrasts in Table 2. There were no differences between parental lines for these traits. Reciprocal effects were significant and of opposite sign, being negative for heterophils and positive for lymphocytes with the negative ratio between them approaching

TABLE 2. Genetic effects on heterophils, lymphocytes, and heterophil:lymphocyte ratios at 30 d of age for males according to contrasts involving various mating combinations of lines HH[1] and LL[1]

Contrast	Heterophils	Lymphocytes	Heterophil:lymphocyte ratio
	(%)		
Parental lines (P)			
HH-LL	17	7	25
Reciprocal effects			
HL-LH	–28*	14*	–36†
Heterosis			
HL-(HH+LL)/2	–25*	12*	35*
LH-(HH+LL)/2	4	–2	1
(HL+LH)-(HH+LL)	–11	5	–17
Maternal heterosis[2]			
HHF_1-F_1HH	4	–2	8
LLF_1-F_1LL	4	2	11
$(4\overline{BC} - 2\overline{F}_2 - \overline{F}_1 - P)/2$	5	2	6
Recombination			
$2(\overline{F}_2 - \overline{F}_1)$	0	0	1
$4(\overline{F}_2 - \overline{BC})$	8	3	–1

[1]HH and LL were selected for high and low antibody response to SRBC, respectively.

[2]HHF_1-F_1HH = (HHHL+HHLH)-(HLHH+LHHH). LLF_1-F_1LL = (LLHL+LLLH)-(HLLL+LHLL).

†$P < 0.06$.

*$P \leq 0.05$.

TABLE 3. Mean hematocrits at 42 d of age by sex and with sexes combined for chicks from all mating combinations of lines HH[1] and LL[1]

Progeny[2]	Male[3]	Female[3]	Sexes pooled[3]
	(%)		
Parental lines and F_1			
HHHH	35.9	36.2	36.0
HHLL	39.2	34.8	37.0
LLHH	34.3	35.3	34.8
LLLL	36.5	36.8	36.6
Backcross to HH parental line			
HHHL	35.2	36.2	35.7
HHLH	35.7	35.7	35.7
HLHH	36.2	36.0	36.1
LHHH	35.0	35.1	35.0
Backcross to LL parental line			
LLHL	35.2	35.0	35.1
LLLH	38.1	34.9	36.5
HLLL	34.0	34.1	34.0
LHLL	36.6	35.8	36.2
F_2			
HLHL	33.3	34.0	33.6
HLLH	32.9	33.0	33.0
LHHL	34.0	34.4	34.2
LHLH	36.2	35.3	35.8
Pooled SEM	0.2	0.2	0.2

[1]HH and LL were selected for high and low antibody response to SRBC, respectively.

[2]The first two letters designate the sire and the second two letters the dam population for the mating combination.

[3]Genetic contrasts are shown in Table 4.

significance ($P < 0.06$). For heterophils and the ratio, values were greater for the LH than the HL cross. In contrast, for lymphocytes the mean was greater for the HL than for the LH cross. Directional heterosis was significant for the HL cross but not the LH cross for heterophils, lymphocytes, and the ratio between them. Heterosis for the HL cross was negative for heterophils and positive for lymphocytes. Neither maternal heterosis nor recombination effects were significant. None of scaling tests was significant with A = –0.28 ± 2.62, 0.28 ± 2.62, and –0.02 ± 0.10, B = 1.91 ± 2.69, –1.91 ± 2.69, 0.05 ± 0.10, and C = –3.97 ± 4.76, 3.97 ± 4.76, –0.15 ± 0.18 for heterophils, lymphocytes, and the heterophil:lymphocyte ratio, respectively.

Hematocrits

Mean hematocrits at 42 d of age are presented for progeny types in Table 3 with genetic contrasts in Table 4. There were no differences in hematocrits between males (35.2 ± 0.2%) and females (35.5 ± 0.2%) nor between parental lines. Reciprocal effects were highly significant for males and when sexes were pooled with values higher for the HL than LH cross. Although heterosis was significant for males but not females of the HL cross, there was no evidence for heterosis for the LH cross. Maternal heterosis was not significant for either sex or when sexes were pooled. Both measures of recombination effects were strongly positive for males and when sexes were pooled, whereas for females there were significant recombination effects in the comparison of the F_2 populations with backcrosses but not for the F_2 with the F_1 generation comparison. Scaling tests were not significant for A and B. Values were: A = 0.016 ± 0.014, 0.002 ± 0.012, –0.007 ± 0.009, B = –0.013 ± 0.014, –0.020 ± 0.012, –0.016 ± 0.009 for females, males, and sexes pooled, respectively. Scaling test C was significant with corresponding values being –0.005 ± 0.025, –0.064 ± 0.022, –0.080 ± 0.017.

DISCUSSION

This study was designed to examine modes of inheritance of heterophils, lymphocytes, and hematocrits under routine husbandry when there were no known stressors. The parental lines differed in response to the SRBC antigen and resistance to several diseases (Gross *et al.*, 1980). The traits measured in this experiment are modified by stressors and disease agents, but information is lacking on genetic variation for them prior to imposition of these environmental insults.

Bayyari *et al.* (1997) reported that lymphocyte numbers were lower in a line of turkeys selected for heavier BW than in a line selected for increased egg production. Biozzi *et al.* (1971) reported higher lymphocyte numbers in spleens of mice selected for high than for low antibody response to SRBC. The lack of differences for heterophils, lymphocytes, and the ratio between them observed in our experiment may be because under our husbandry there was an optimum level of stress where the ratio was about 0.5. Such values were in contrast to

TABLE 4. Genetic effects for hematocrits at 42 d of age by sex and sexes combined according to contrasts involving various mating combinations of lines HH[1] and LL[1]

Contrast	Male	Female	Sexes pooled
	(%)		
Parental lines (P)			
HH-LL	–2	–2	–2
Reciprocal effects			
HL-LH	14**	–1	6**
Heterosis			
HL-(HH+LL)/2	8**	–5	2
LH-(HH+LL)/2	–5	–3	–4
(HL+LH)-(HH+LL)	2	–4	–1
Maternal heterosis[2]			
HHF_1-F_1HH	–1	1	<1
LLF_1-F_1LL	4	0	2
$(4\overline{BC} - 2\ \overline{F}_2 - \overline{F}_1 - P)/2$	1	1	1
Recombination			
$2(\overline{F}_2 - \overline{F}_1)$	–7**	–2	–5**
$4(\overline{F}_2 - \overline{BC})$	–5**	–3*	–4**

[1]HH and LL were selected for high and low antibody response to SRBC, respectively.

[2]HHF_1-F_1HH = (HHHL+HHLH)-(HLHH+LHHH). LLF_1-F_1LL = (LLHL+LLLH)-(HLLL+LHLL).

*$P < 0.05$.

**$P \leq 0.01$.

ratios of <0.2 and >0.8, which indicate low and high levels of stress, respectively (Gross and Siegel, 1993).

Heterophils have been reported to phagocytose and digest *Escherichia coli*, *Bacillus megaterium*, and *Staphylococcus aureus* (Gross, 1962). Even though Line LL resisted these pathogens better than Line HH (Gross *et al.*, 1980), there was no line difference in number of heterophils in this experiment, in which the chickens were not infected with these organisms. This result may be because alterations in numbers of heterophils that may be line-specific occur by infection or stressors (Gross and Siegel, 1983) and suggests that genetic variation may exist in the immunocompetence of heterophils. The modes of inheritance of spleen weight, before and after infection, in these selection lines were also different (Boa-Amponsem *et al.*, 1998).

Divergent selection for antibody response to SRBC did not result in a correlated response in hematocrits, which may be an example of stabilized selection in these White Leghorns. Broilers may benefit from intermediate hematocrit values, as shown by Shlosberg *et al.* (1996), who reported that selection for high hematocrits was associated with an increased incidence of ascites whereas mortality from causes other than ascites was greater when selection was for lower hematocrits. In this experiment, the lack of sexual dimorphism for hematocrits was consistent with the research of Washburn and Siegel (1963) in which in lines selected for BW, values for males were similar to those for females until sexual maturity. Also, Shlosberg *et al.* (1992) did not observe sexual dimorphism for hematocrits at 42 and 49 d of age in a fast-growing strain of broilers.

Although sex-linked and maternal effects may influence reciprocal effects, maternal, but not sex-linked effects, should affect both sexes similarly. For hematocrits, analyses for sexes showed that sex-linkage was important. Also, recombination effects appeared to be important for hematocrits, as evidenced by measures unbiased by maternal heterosis as well as the significant scale test C. The effects, however, were probably a reflection of the sex-linkage for this trait.

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