Antibody Response of Chickens to Sheep Red Blood Cells: Crosses Among Divergently Selected Lines and Relaxed Sublines

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ABSTRACT Crosses were made among lines of chickens that had undergone 30 generations of selection for high or low antibody response 5 d after an intravenous injection with SRBC, and between sublines in which selection was relaxed in generation 24. Antibody responses at 5, 10, and 14 d after injection were measured in the 4 lines and in reciprocal crosses among them. Divergence between the high and low lines selected for SRBC antibody was immediate and increased during selection. Although significant in both cases, separation of the relaxed subline from its respective selected line was greater in the high than the low line. Five-day SRBC titers of the relaxed lines and the crosses were intermediate to the high and low selected lines, with the direction and magnitude of heterosis being line dependent. A high proportion of chickens from low line mating combinations did not have detectable antibody titers at 10 and 14 d postinoculation with SRBC, precluding statistical analysis of these data. Results are discussed in the context of intra- and interlocus effects on the selected trait.

Key words: chicken, heterosis, selection, sheep red blood cell, genetic variation

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INTRODUCTION

Heritability of antibody response of chickens to an inoculation of SRBC is moderate and the trait responds to divergent selection (Siegel and Gross, 1980; van der Zijpp and Leenstra, 1980; Martin et al., 1990; Pinard et al., 1992). Concomitant with the direct response of SRBC antibody titers to selection are correlated responses in production and disease-related traits (see reviews by Pinard-van der Laan et al., 1998; Lamont et al., 2003). The literature is inconsistent concerning the role of nonadditive genetic variation in antibody responses to SRBC (Siegel et al., 1982; Ubosi et al., 1985; Pinard and van der Zijpp, 1993; Boa-Amponsem et al., 1997). The experiment reported here was designed to compare the effects of crossing lines divergently selected for 30 generations for high or low response to SRBC with each other and with sublines in which selection had been relaxed for 8 generations.

MATERIALS AND METHODS

The Populations

Chickens used in this experiment were White Leghorns from 2 lines selected for 30 generations for high (**HS**) or low (**LS**) antibody response 5 d after a single intravenous injection of 0.1 mL of a 0.25% suspension of SRBC (Siegel and Gross, 1980; Martin et al., 1990). In generation 24, 10 males and 20 females were selected at random from the HS and LS lines to form sublines in which selection was relaxed. These high relaxed (**HR**) and low relaxed (**LR**) lines have been reproduced and maintained as contemporaries with the selected lines. The selected lines were pedigreed with 8 males, each mated to 4 females. The relaxed lines were reproduced using pooled semen with 10 males and 20 females.

The Matings and Husbandry

The present experiment involved progeny from 12 mating combinations of S_{30} generation selected and R_7 generation relaxed lines. Matings by sire × dam combinations were HS × HS, HR × HR, LR × LR, LS × LS, HS × HR, HR × HS, LS × LR, LR × LS, HR × LR, LR × HR, HS × LS, and LS × HS. Eggs were obtained from age-contemporary parents and incubation was in the same incubator and hatcher. Upon hatching, chicks were wing-banded, vaccinated for Marek's disease, and placed in pens with wood shavings as litter. They were provided ad libitum a mash diet of 20% CP and 2,685 kcal of ME/kg.

At 44 d of age, a minimum of 10 males and 10 females from each mating combination were given a single injection of 0.1 mL of a 0.25% suspension of SRBC via the brachial vein. Then, 5, 10, and 14 d later, a sample of approximately 0.5 mL of blood was obtained from the brachial vein of each individual and transferred into a tube containing 2 drops of EDTA. After refrigeration (to allow the red blood cells to settle), plasma antibodies were

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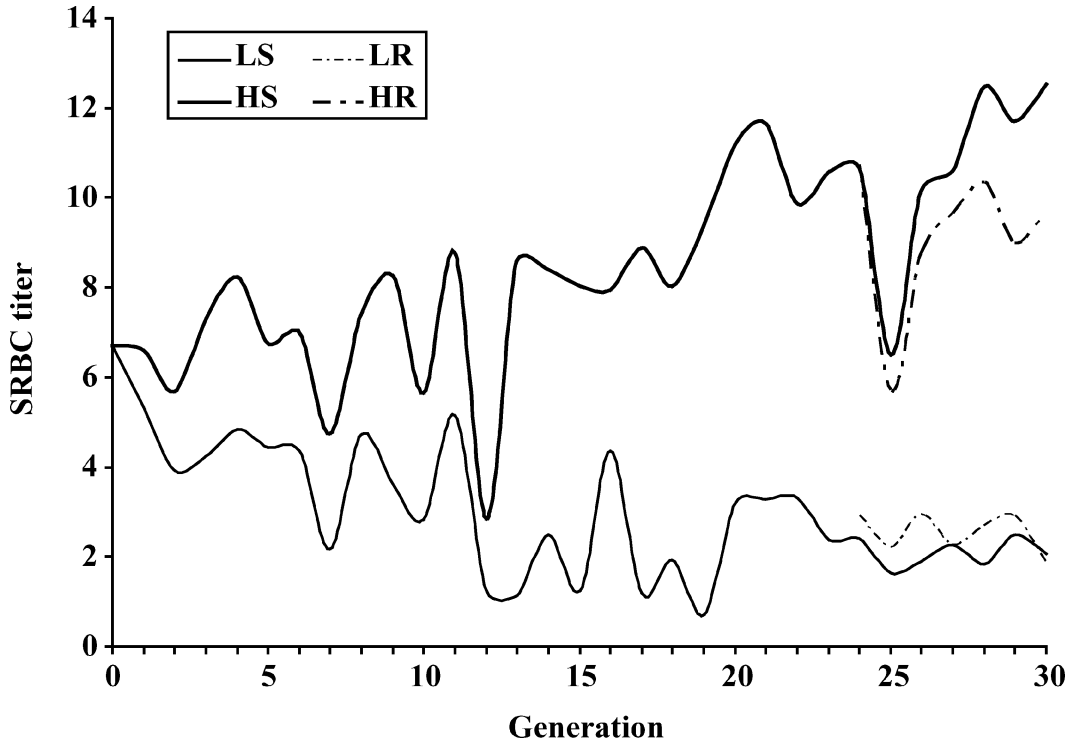

Figure 1. Antibody titers of male chickens 5 d after an i.v. injection of 0.1 mL of a 0.25% suspension of SRBC. There were 30 generations of selection for high (HS) or low (LS) titers. Relaxed sublines HR and LR were initiated in generation 24 and reproduced at random.

measured by the microtiter hemagglutination method of Wegmann and Smithies (1966). Titers were expressed as log2 of the reciprocal of the highest dilution in which there was hemagglutination.

Analyses

Five-day titers (y_{ijk}) were analyzed by an ANOVA model:

$$y_{ijk} = \mu + s_i + l_j + (sl)_{ij} + e_{ijk}$$

where μ was the overall mean, s_i was the sex of individual k, l_j was the line cross used to produce individual k, $(sl)_{ij}$ was the sex by line interaction, and e_{ijk} was the random residual error. Reciprocal crosses were combined into single subclasses because previous research with these lines (Boa-Amponsem et al., 1997) had indicated no significant maternal or paternal effects. Least squares means were derived for line and line × sex interaction effects. Heterosis was calculated and tested for significance for each cross using contrasts comparing the midparent average of the lines to the average of their crosses.

Due to the number of individuals in the LS and LR lines without detectable antibody titers on d 10 and 14 postinoculation, variances were heterogeneous across line subclasses. As a result, these titers were not statistically analyzed.

RESULTS AND DISCUSSION

The Lines – A General Description

Divergence between the selected lines for SRBC antibody was immediate (Figure 1) and increased such that there was little overlap in their distributions after 14 generations (Martin et al., 1990). Although this response to individual phenotypic selection implied considerable additive genetic variation for the trait, crosses made periodically between the selected lines (Siegel and Gross, 1980; Siegel et al., 1982; Boa-Amponsem et al., 1997) reflected some nonadditive genetic variation.

Responses of lines HS and LS over the 30 generations of selection were irregular and characterized by cessations of response followed by further responses. Such patterns or "waves of response" are not uncommon in long-term selection experiments and may reflect spontaneous mutations assimilated into a line as well as interlocus dynamics, which Eitan and Soller (2004) termed "selection-induced genetic variation." Such variation has been observed in our lines of chickens that had undergone long-term selection for BW at 56 d of age (Carlborg et al., 2006). During the course of this selection experiment for antibody response to SRBC, attempts were made to avoid major environmental effects across generations (except that the source of SRBC was a different sheep each generation) and the shifts appeared to influence all lines. No explanation for these swings is apparent; however, their parallel nature implies environmental effects.

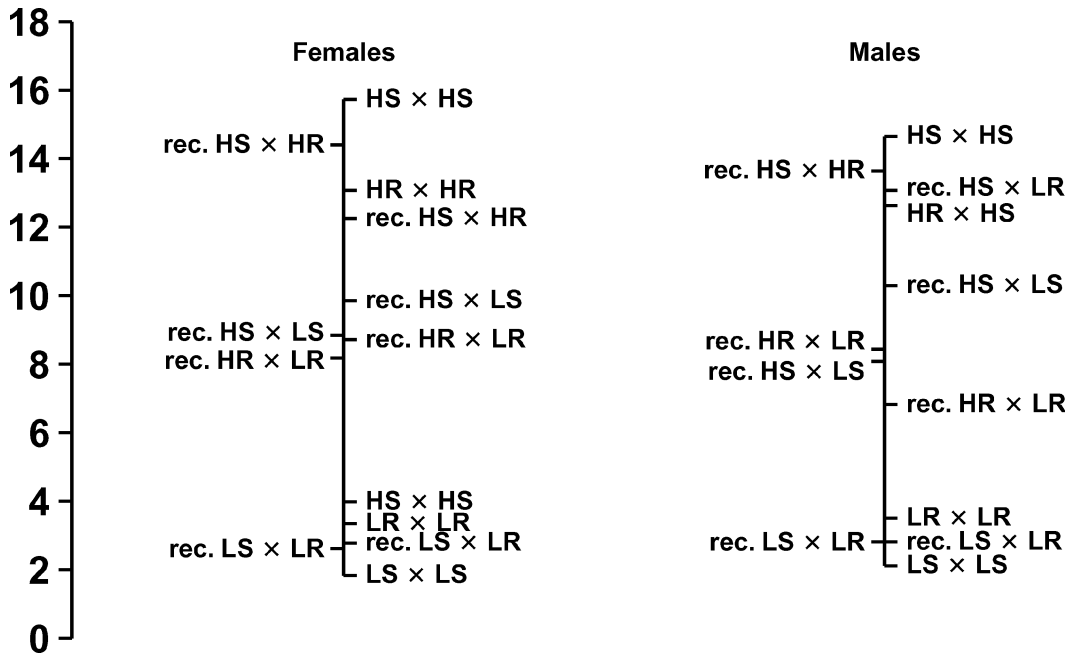

Figure 2. Antibody titers of female and male chickens 5 d after an i.v. injection of 0.1 mL of a 0.25% suspension of SRBC, by mating combination. Left of vertical lines are the expected midparent values and right of vertical lines are the actual values. rec. = reciprocal crosses combined; HS = high antibody select; HR = high antibody relaxed; LR = low antibody relaxed; LS = low antibody select.

Fluctuations appeared more dramatic in the HS than in the LS line. This difference is not surprising because the LS line appears to have reached a plateau. The plateau may be due, in part, to having reached a threshold for antibody response to the dosage of SRBC inoculated. Previous experiments with these lines have shown that thresholds for antibody response to SRBC are influenced by dosage (Ubosi et al., 1985) and route of administration (Boa-Amponsem et al., 2001).

When selection was relaxed in generation 24, there was some evidence for a return to higher antibody levels in the LR line (Figure 1). In contrast, the HR line, although following the generation-to-generation pattern of the HS line, showed considerable regression to the origin suggesting nonadditivity in the high direction, which was consistent with the heterosis reported previously (Siegel et al., 1982; Boa-Amponsem et al., 1997). Perhaps more importantly, the results are consistent with a growing body of data that suggest an intermediate optimum for antibody response (see review by Lamont et al., 2003). For the lines reported here and the crosses between them, Siegel et al. (1982) and Martin et al. (1990) proposed that the negative relationships between SRBC antibody responses and production traits suggested that natural selection favored an intermediate immune response. This was because over-production of antibodies had a negative effect on fitness relative to other traits. Gross et al. (2002) took this reasoning further by challenging these lines and the crosses between them with a range of challenging agents. The defense mechanisms were not only resource expensive, but depending on the genetic mechanisms involved, the populations were reranked depending on the challenging agent.

Crosses and Parental Line Comparisons

Five-Day Titers. Sex × mating combination interactions for antibody titers 5 d after injection with SRBC were significant. Thus, comparisons of mating combinations are presented separately for males and females (Figure 2). As expected, progeny from the HS and LS lines were different and formed "bookends" for the other mating combinations. Antibody titers were lower ($P < 0.01$) for HR than HS progeny and higher ($P < 0.01$) for LR than LS progeny. These differences between the respective selected and relaxed lines are consistent with those noted earlier in this paper. That is, although response to selection appears to have continued in the H line, confounding exists whereby relaxing selection in the H line resulted in a regression toward the origin. This pattern was not observed in the L line.

The reciprocal HL cross differed from the parent lines in both sexes. When obtained values were compared with their expected midparent values, they were higher for females ($P < 0.1$) and for males ($P < 0.01$). This pattern again reflected the influence of nonadditive genetic variation with heterosis being 10 and 17% for females and males, respectively; these values are consistent with those reported for earlier generations (Siegel and Gross, 1980; Siegel et al., 1982; Boa-Amponsem et al., 1997). These results would suggest involvement of loci on the sex chromosomes.

Comparisons between expected and actual SRBC antibody titers for the crosses between lines in which selection was relaxed were similar for females but lower for males ($P < 0.05$), suggesting that the regression toward the origin from relaxation in the H line reduced heterozygous com-

Table 1. Mean antibody responses 10 and 14 d after i.v. inoculation of SRBC by mating combination[1] and sex

	10 d		14 d	
Mating	Male	Female	Male	Female
Parental lines				
HS × HS	8.9	8.8	5.6	5.6
HR × HR	4.4	5.2	3.0	4.0
LR × LR	1.7	2.2	1.3	1.3
LS × LS	1.3	1.5	1.1	1.1
Reciprocal crosses				
HS × HR	6.6	6.6	4.6	4.7
HR × LR	3.8	5.1	2.9	3.8
HS × LS	5.9	5.8	3.8	3.8
LS × LR	1.4	1.8	1.4	1.4

[1]HS = high antibody select; HR = high antibody relaxed; LS = low antibody select; LR = low antibody relaxed.

binations for loci associated with higher antibody response to SRBC. This reasoning was further reinforced by comparisons involving crosses between the selected lines and their relaxed counterparts. Antibody titers of progeny from matings between LS and LR were intermediate to those of their parental lines and essentially the same as expected midparent values. In contrast, for the H lines, antibody titers of the crosses were closer to those for the HR than the HS line with a difference ($P < 0.001$) between actual and expected values with negative heterosis consistent with the selection-induced genetic variation thesis of Eitan and Soller (2004) and an intermediate optimum.

Ten- and Fourteen-Day Titers. Antibody titers 10 and 14 d after injection with SRBC are summarized in Table 1. These data were not analyzed statistically because of the high proportion of progeny from LR × LR, LS × LR, LR × LS, and LS × LS matings that no longer had detectable antibody titers to SRBC. As seen in Table 1, there was a consistent trend from the HS to the LS parental line, which followed that noted for titers 5 d after inoculation.

General Comments. Maintenance of genetic and phenotypic plasticity in a long-term selection experiment such as this one implies intra- and interlocus sensitivity to environmental factors as well as artificial selection per se. The parallel "waves of response" noted within and across generations reflect phenotypic plasticity to environmental factors. That these factors did not mask continued responses to selection at the individual phenotypic level and that there was evidence of modest heterosis suggests additivity and dominance at individual loci as well as interlocus sensitivity (networks of pleiotropic genes). Subsequent studies may provide further insights into such relationships among loci (Carlborg et al., 2006) per se as well as the interface of genetic and phenotypic plasticity in lines such as these in which selection continues and is relaxed.

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