Genetic Parameters for Antibody Response of Chickens to Sheep Red Blood Cells Based on a Selection Experiment

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ABSTRACT In the present study, a selection experiment for antibody (Ab) titer against SRBC in chickens was analyzed. Two lines were divergently selected for increased and decreased Ab titer. Further, a randombred control line, originating from the same base population, was included in the experiment. The heritability for immune response against SRBC was estimated after 18 generations of selection. In total, Ab titers obtained from 16,459 chickens were included in the analysis. Data was analyzed using an animal model. Posterior distributions for variance components and heritability were obtained using Markov Chain Monte Carlo methods. Response to selection was evaluated by constructing the posterior distribution for the genetic trend. In addition, a bivariate animal model was used in which male and female antibody titers were treated as different traits to estimate correlations between male and female immune response against SRBC. The heritability of Ab titer response, when using information from all three lines, was 0.18. The 90% highest posterior density region for the estimated heritability ranged from 0.16 to 0.19. In Generation 18, the genetic difference between the high and the low line was 5.1 phenotypic standard deviations. Analyses of each line separately revealed large differences in heritabilities between the lines, which could be mainly attributed to differences in error variances between the lines. The results suggest that selection for high Ab titers cause an increased environmental sensitivity. The estimated genetic correlation between male and female Ab titer was 0.92 and was not significantly different from 1.

(*Key words*: chicken, selection experiment, heritability, sheep red blood cells, antibody titer)

2002 Poultry Science 81:309–315

INTRODUCTION

Production of poultry meat and eggs has intensified drastically in the last few decades, not in the least because of decreasing margins between costs and income. These developments have increased the risk of infectious diseases (Kreukniet, 1995). Several studies have shown that a positive correlation exists between certain production traits and diseases, e.g., between body or egg weight and mortality due to Marek's disease (Gavora, 1990; Pinard et al., 1993). These results suggest an increase in disease susceptibility if selection is only for particular production traits (Pinard et al., 1993). In the long term, this selection might lead to increased mortality, production losses, and an increase in the use of medication (Dunnington et al., 1992). Besides the economic benefits from improving disease resistance, the possibility of reducing medication would be an attractive feature from standpoints of public health, ethics, product quality, and animal welfare (Pinard et al., 1993). Improvement of disease resistance might be possible by selecting poultry for increased immune response.

Pinard et al. (1992a) estimated the heritability for Ab titer against SRBC based on nine generations of divergent selection. At present, information from this selection experiment is available for 18 generations; thus enabling a more accurate estimation of the heritability and an evaluation of the present genetic differences between the lines. Differences exist in antibody titers between males and females; hens have higher antibody titers against SRBC then cocks (Siegel and Gross, 1980; Leitner et al., 1989; Parmentier et al., 1996). This difference might be due to male and female antibody titers being genetically different traits, due to the genes being located on the sex chromosome. This possibility can be studied by estimating the genetic correlation between male and female antibody titers. The aim of the current study was to estimate the heritability for immune response against SRBC in selected lines, estimating the genetic trend and genetic correlation

Received for publication January 22, 2001.
Accepted for publication November 5, 2001.
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Abbreviation Key: Ab = antibody, HPD = highest posterior density.

TABLE 1. Number of animals with observations per generation and per line

Generation	High	Control	Low	Total
0	. . .	. . .	. . .	614
1	289	248	215	752
2	278	242	215	735
3	298	332	317	947
4	327	230	317	874
5	207	266	236	709
6	372	196	395	963
7	347	248	374	969
8	329	262	353	944
9	411	269	430	1,110
10	146	160	155	461
11	247	215	313	775
12	344	180	271	795
13	367	274	363	1,004
14	280	304	249	833
15	443	178	428	1,049
16	364	299	311	974
17	324	286	327	937
18	371	279	364	1,014
Total	5,744	4,468	5,633	16,459

between male and female immune responses against SRBC.

MATERIALS AND METHODS

Data

The data set for this experiment were from 16,459 chickens, which have phenotypic observations on Ab titer against SRBC. Selection started in 1981 from a base population of 614 birds (Generation 0) and has continued for 18 generations. Two lines have been divergently selected for increased (high line) and decreased (low line) Ab titer. Selection was based on the individual total antibody titer 5 d after the primary intramuscular immunization at 37 d of age with 1 mL of 25% SRBC diluted in PBS (Pinard et al., 1992a). Total antibody titers to SRBC were determined by agglutination with routine procedures (Van der Zijpp and Leenstra, 1980). Antibody titers measured against SRBC were expressed as the $\log_2$ of the reciprocal of the highest plasma dilution giving complete agglutination. All titrations were assessed the same day in 96-well microtiter plates, using erythrocytes from the same sheep that was used to immunize chickens.

Besides the high and low lines, a randombred control line, originating from the same base population, was included in the experiment. Males from each generation were assigned randomly to females, avoiding the mating of full sibs. Generations were not overlapping. Table 1 shows the number of individuals with Ab titers per generation and per line.

In the high and low lines, approximately 25 males and 50 females were selected from each generation. In the control line, approximately 40 males and 70 females were randomly chosen as parents for the next generation. The selected chickens were randomly mated, but mating of full and half-sibs was avoided.

Estimating Heritabilities

Heritabilities were estimated using a single-trait animal model. The following mixed linear model was used:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

where $\mathbf{y}$ = n × 1 vector of observations on Ab titer; $\mathbf{X}$ and $\mathbf{Z}$ = known incidence matrices related to fixed effects and random additive genetic effects, respectively; β = vector of fixed effects, including generation (18 levels) and sex; $\mathbf{u}$ = vector of random additive genetic effects; and $\mathbf{e}$ = vector with random residuals. Assumptions with respect to the distributions of random effects were as follows:

$$\mathbf{u} \sim N(0,\mathbf{A}\sigma_a^2) \text{ and } \mathbf{e} \sim N(0,\mathbf{I}\sigma_e^2)$$

where σ_a^2 = additive genetic variance, σ_e^2 = error variance, $\mathbf{A}$ = additive genetic relationship matrix, and $\mathbf{I}$ = identity matrix. The base population consisted of 116 parents of individuals in Generation 0 and is indicated as Generation −1.

A Markov Chain Monte Carlo method (Janss et al., 1995), from the MAGGIC software, was used for the single-trait analysis. The Gibbs sampler was used to generate samples from a joint posterior density (e.g., Gelfland and Smith, 1990). Based on these samples, the marginal posterior densities for variance components, heritability, and genetic trend were studied. In the algorithm, sampling is applied to all unknown parameters in the model, as described in more detail by Janss et al. (1995). Flat priors were used for variance components and for fixed effects. One long Markov chain is produced for statistical inference by repeating the updating scheme. Consecutive realizations show serial correlations, which are removed by thinning. Further, the Markov chain requires several iterations before the equilibrium distribution is reached, i.e., the burn-in period. In the present analysis, the thinning

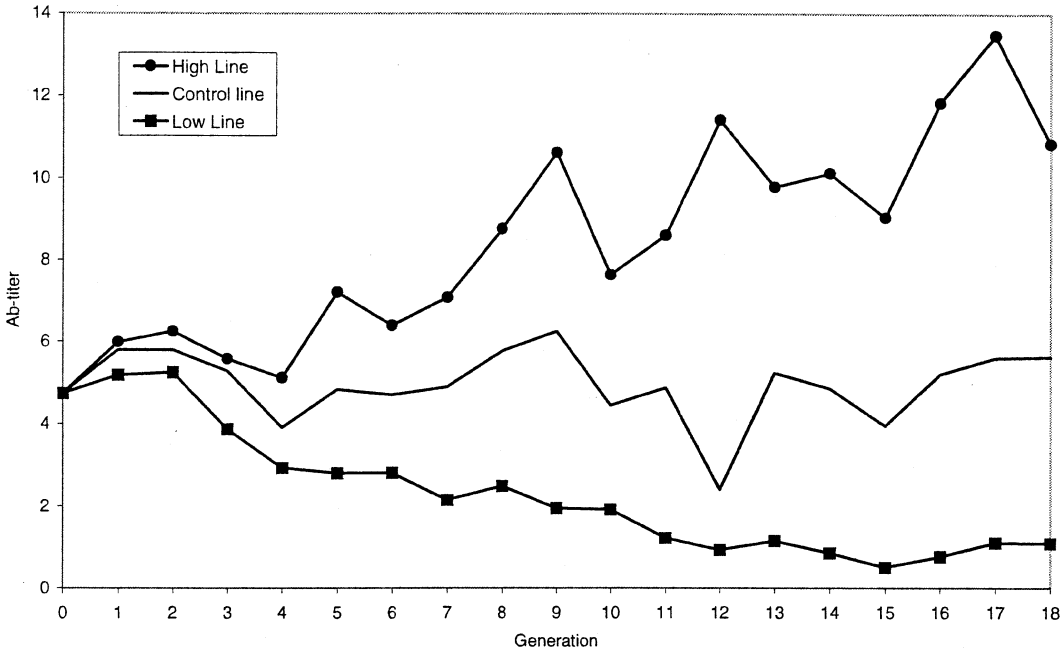

FIGURE 1. Mean phenotypic values for antibody titer in the high, control, and low lines.

parameter was 100 and the burn-in was 5,000. The chain was run for 505,000 cycles leaving 5,000 samples for constructing posterior distributions. Response to selection was evaluated by creating the posterior distribution for the genetic trend, which was obtained by averaging the estimated breeding values of all individuals in a certain line-by-generation combination.

A bivariate animal model was used in which male and female Ab titers were treated as different traits. The model is similar to the single-trait model, except that the fixed effect of sex was excluded. Solutions for this model were obtained by using the computer package ASREML (Gilmour et al., 1999).

Genetic trends and trends for inbreeding were estimated using regression. The following model was used:

$$y_i = \mu + bx_i + e_i$$

where $\mathbf{y}_i$ = average inbreeding coefficient or average breeding value of chickens in one of the three lines in generation i, μ = mean, b = regression coefficient, $\mathbf{x}_i$ = Generation i, and $\mathbf{e}_i$ = random residual.

RESULTS

Phenotypic Trend

Figure 1 shows the mean phenotypic level for Ab titer of the three lines in each generation. The average Ab titer of chickens in Generation 0 was 4.7. The mean Ab titer of the control line had a maximum value of 6.2 in Generation 9 and a minimum Ab titer of 2.4 in Generation 12. In Generation 18, the Ab titer for the control line was 5.6, which was somewhat higher than the value of 4.7 in Generation 0. The high line showed considerable fluctuations in Ab titer from one generation to the next, e.g., in Generation 17 the average Ab titer was 13.5 and in Generation 18 it was 10.8. The overall picture showed a continuing increase of the Ab titer for the high line. For the low line, a steady decrease of Ab titer was observed from Generations 2 to 12 (average Ab titer was 0.9). After that generation there was no further systematic decrease in Ab titer.

Inbreeding Coefficient

Figure 2 shows the average inbreeding coefficient for the three selection lines. The increase in inbreeding coefficient was very similar for the high and low lines. As expected, the average inbreeding coefficient was lower for the control line. In Generation 18 the average inbreeding coefficient was 17.3% in the high line, 16.3% in the low line, and 7.0% in the control line. The increase of inbreeding per generation, as estimated by linear regression, was 0.96% for the low line, 0.86% for the high line, and 0.40% for the control line.

Genetic Parameters

When using information from all three lines, the estimated heritability was 0.177. The 90% highest posterior density (HPD) region for the estimated heritability ranged from 0.163 to 0.190 (Figure 3). The estimated additive genetic variance was 0.838, and the error variance was 3.904. The estimated genetic trend and its 90% HPD region, based on analysis of all three lines simultaneously, are shown in Figure 4. The mean genetic value of the base population (Generation –1) was 0. The genetic level of the control line increased slightly to +0.7 in Generation 18. In the high line, the mean genetic value increased to

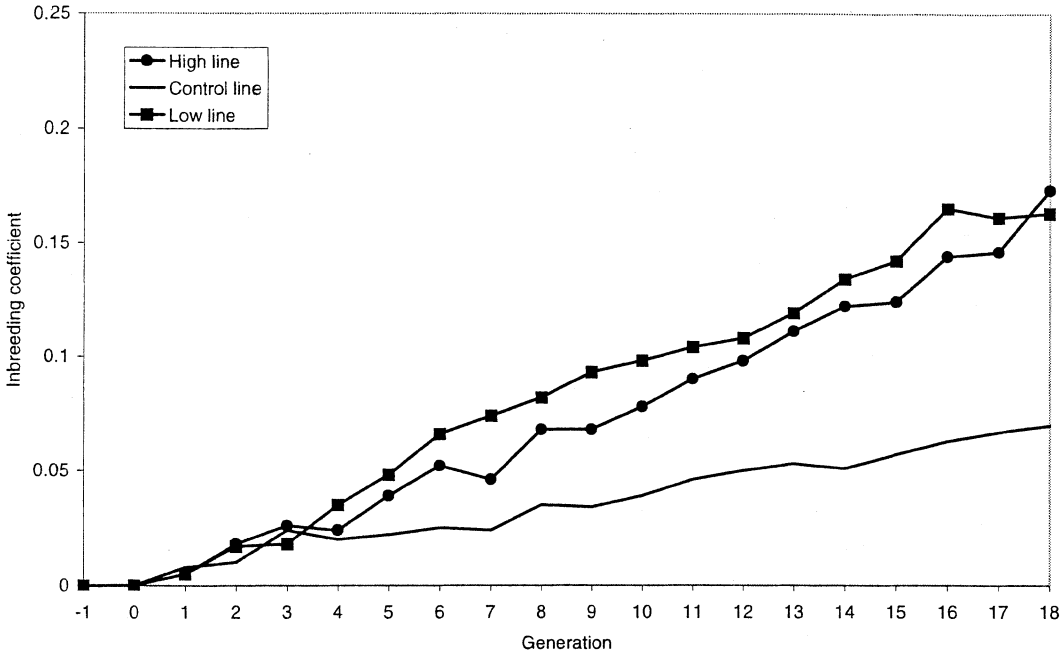

FIGURE 2. The average inbreeding coefficient in each generation for the high, control, and low lines.

+7.3 in Generation 18. Fitting a linear regression results in an estimated genetic response of +0.41 per generation. For the low line, the genetic trend showed a steady decrease until Generation 9, and from there on the genetic level stayed more or less constant. Fitting a linear regression line through the first nine generations resulted in a decrease of Ab titer of 0.42 per generation. This response was similar to that of the high line.

To detect differences between upward and downward selection, analyses were performed for each line separately. However, in those analyses, genetic and generation effects could not be separated. Therefore, the observations were pre-adjusted for generation effects that were obtained when analyzing all three lines simultaneously. The data in Table 2 shows the results of these analyses. The estimated heritabilities between the three lines differ considerably. This variation appears to be mainly due to differences in the estimated error variance. Although estimated heritability in the high line was lower than in the control and low lines, additive genetic variance in the

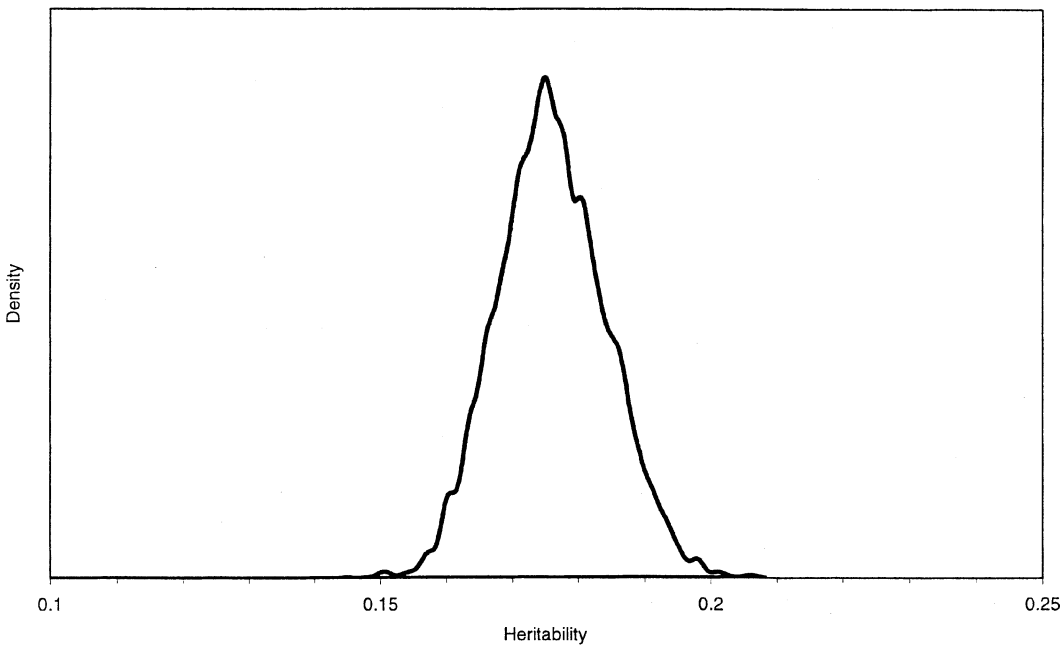

FIGURE 3. Posterior distribution for the heritability of antibody titer against SRBC when analyzing all three lines simultaneously.

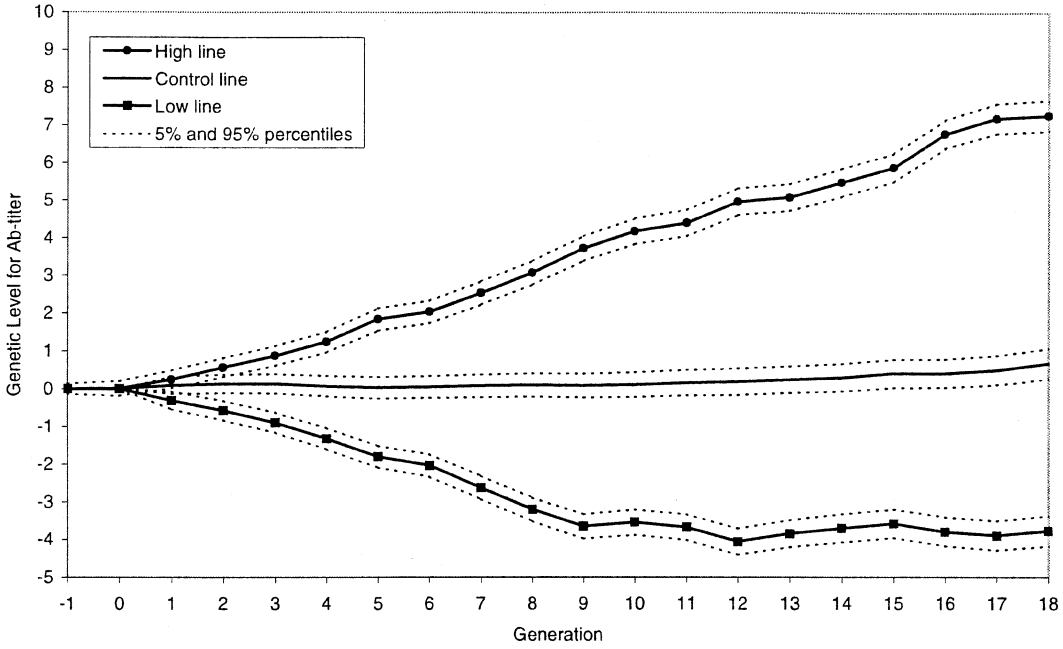

FIGURE 4. Genetic trends for antibody titers and their 90% highest posterior density region for the high, control, and low lines.

high line was greater than in the two other lines. The error variance in the high line was 6.031, which was considerably higher than the error variances in the control (3.003) and the low (1.813) lines.

The data in Table 3 show the estimated additive genetic variance when including data from Generations 1 to 9 or from Generations 12 to 18 in the analyses. Results are shown separately for each of the three selection lines, and observations were pre-adjusted for generation effects. For the high and low lines, the additive genetic variance was lower in later generations than in earlier generations. However, for the low line only, the 90% HPD regions of both estimates did not overlap. For the control line, the estimated additive genetic variance in later generations was higher, but differences were not significant. Note that the 90% HPD regions in Table 3 are somewhat underestimated, as in these analyses generation effects were assumed to be without error.

The bivariate analysis resulted in a heritability estimate of 0.18 for male Ab titer and 0.19 for female Ab titer. The estimated genetic correlation between male and female Ab titer was 0.92. Standard error of the estimated genetic correlation was 0.05.

TABLE 2. Estimates of heritabilities and variance components for each line separately when pre-adjusting the data for generation effects

Line	h^2	σ_a^2	σ_e^2
High	0.151	1.072	6.031
Control	0.203	0.764	3.003
Low	0.276	0.692	1.813

DISCUSSION

Literature

Martin et al. (1990) found heritabilities of 0.25 in a selection line for increased Ab titer to SRBC and 0.23 in a selection line for low Ab titers to SRBC. Selection lines were between Generations 10 and 14. Siegel and Gross (1980) reported a heritability for Ab titer to SRBC of 0.44 for high-line males, 0.17 for high-line females, 0.19 for low-line males, and 0.26 for low-line females. Except for the high heritability for high-line males reported by Siegel and Gross (1980), all of these studies point to a heritability for Ab titer to SRBC of 0.20 to 0.25, which is supported by the findings in the present study. For IgM and IgG levels after SRBC stimulation, Sarker et al. (1999) reported higher heritabilities of 0.61 and 0.52, respectively. The Ab titers in the present selection experiment are largely of the IgM type.

By using reciprocal crossings, Boa-Amponsem et al. (1997) found that the average Ab titer to SRBC in female offspring from high-line females and low-line males was higher than that of female offspring from low-line females and high-line males. This finding is an indication that there are genes affecting Ab titer against SRBC located on the sex chromosome of the chicken genome. The genetic correlation of 0.92 with a standard error of 0.05 reported in the present study was not significantly different from one. Therefore, results found by Boa-Amponsem et al. (1997) could not be confirmed in the present study.

The present study shows a progressive increase of Ab titers to SRBC in the high line as well as a progressive decrease in the low line with a heritability of 0.18. Selection has resulted in divergent Ab responses to other anti-

TABLE 3. Estimated additive genetic variance based on information from Generations 1 to 9 or Generations 12 to 18

	Generations	
Line	1–9	12–18[1]
High	1.297 (1.123–1.477)[2]	0.992 (0.745–1.278)
Control	0.669 (0.500–0.852)	1.004 (0.693–1.364)
Low	0.873 (0.760–0.996)	0.560 (0.430–0.699)

[1]Base generation consisted of chickens from Generation 11.
[2]90% highest posterior density region.

gens as well (Parmentier et al. 1994, 1996), although titers in the low line to these antigens do not equal zero. These results suggest that selection has affected the antigen-specific immune response as well as the antigen-nonspecific immune response.

Gibbs Sampling

In the present study, a Bayesian analysis was used to analyze the results of selection for Ab titer against SRBC. As indicated by Wang et al. (1994a,b), this type of analysis enables the estimation of genetic response while taking into account uncertainty about all other parameters, including the variance components. Classical estimators of genetic change using the animal model strongly depend upon the variance ratios of random effects (Sorensen et al., 1994). Results from the Bayesian analysis using flat priors were compared with univariate analysis using ASREML. The heritability estimates of both analyses were identical; however, the classical analysis only results in point estimates.

Analysis of Each Line Separately

All three selection lines were analyzed simultaneously in the present study. When analyzing each line separately, genetic and generation effects could not be separated. As a consequence of this confounding, analyses resulted in very high heritability estimates ($h^2 = 0.41$ for the low line) and an overestimation of the genetic trend. The nonoverlapping generations in the current experiment did not allow separation of genetic and generation effects. These problems do not occur if all three or two of the lines are analyzed simultaneously. Therefore, in the analysis of the separate lines, phenotypic observations were pre-adjusted for generation effects.

Development of Additive Genetic Variance

Sorensen and Kennedy (1984) showed that the use of a full animal model will account for drift and selection and will allow estimation of additive genetic variance in the base population. The underlying assumption of the methodology, however, is the infinitesimal model, i.e., many unlinked additive genes for which frequencies do not change as a result of selection. This assumption might be violated for long-term selection experiments (Meyer and Hill, 1991). Based on data from Generations 1 to 9, Pinard et al. (1992) estimated a heritability of 0.31, which is considerably higher than the estimate of 0.18 found in the present study. In both studies, heritabilities were estimated for the same base population and, therefore, were expected to be the same except for differences due to sampling.

Heritability estimates in the current study were also performed after each generation, i.e., including all information of previous generations. In this way, development of heritability estimates could be monitored. Results indicated an increase of heritability until Generation 9, and, after that, heritability estimates decreased. In Generation 9 the estimated heritability reached a maximum value of 0.31, i.e., the heritability reported by Pinard et al. (1992a). The decrease after Generation 9 might be due to fixation of genes and the consequential decrease of the additive genetic variance. Estimates based on data from Generations 12 to 18, taking chickens in Generation 11 as the base population, showed decreased additive genetic variance in the high and the low lines. However, the additive genetic variance in Generation 11 was expected to be reduced due to selection and inbreeding. Given the mean inbreeding coefficients, intensities of selection, and a heritability of 0.20, the additive genetic variance in Generation 11 of the high and the low selection lines was expected to be approximately 80% of the additive genetic variance in Generation 0, i.e., 1.038 for the high line and 0.698 for the low line. Therefore, the results for the high line can be explained by selection and inbreeding. For the low line, the estimated additive genetic variance in Generation 11 (0.560) and the expected additive genetic variance (0.698), after accounting for selection and inbreeding, do not differ significantly. Therefore, it cannot be concluded that the additive genetic variance in the low line was reduced due to changes in allele frequencies.

Development of Environmental Variance

In the high line, phenotypic variance increased over time. Analyses show that this increase was mainly due to an increased error variance. This change of variance with the mean (scale effects) can be removed by applying a transformation that might remove or reduce variance attributed to epistatic interaction or to interaction between the genotype and the environment (Falconer, 1989). Ab titers used in the present study are expressed on a log-scale, but apparently, this scale could not remove all the differences in error variances between lines. The increased error variance in the high line suggests that selection for high Ab titers resulted in an increased environmental sensitivity. This phenomenon is difficult to explain but has been previously observed in other selection experiments (Meyer and Hill, 1991).

Selection Limit in the Low Line

There was no further decrease of the genetic level in the low line after Generation 9, which suggested that a

selection limit had been reached. However, data from Generations 12 to 18 indicate that the additive genetic variance in the low line is significantly different from zero and, therefore, did not support the presence of a selection limit. The lack of selection response can, in part, be explained by the reduced realized selection differential: the realized selection differential in the last generations is half the selection differential that was realized in the first generations. The selection differential is reduced because, in the last generations, over 50% of the individuals in the low line had an Ab titer of 0. A further decrease of the genetic level in the low line might be achieved if the analytical method used to determine Ab titers is refined to make it possible to distinguish among individuals that currently have Ab titers of 0, which might be possible by using solid-based antibody detection systems (ELISA, RIA).

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