SELECTION FOR IMMUNORESPONSIVENESS IN CHICKENS:

EFFECTS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

AND RESISTANCE TO MAREK'S DISEASE.

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SELECTION FOR IMMUNORESPONSIVENESS IN CHICKENS: EFFECTS OF THE MAJOR HISTOCOMPATIBILITY COMPLEX AND RESISTANCE TO MAREK'S DISEASE.

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Pinard, M.-H., 1992. Selection for immunoresponsiveness in chickens: effects of the major histocompatibility complex and resistance to Marek's disease (Selectie op immuunrespons bij kippen: effecten van het major histocompatibiliteits-complex en resistentie tegen de ziekte van Marek). Improving genetic disease resistance may be an attractive preventive measure in the control of infectious diseases in livestock production. Chickens were selected for high and low antibody response to sheep red blood cells for ten generations. Divergent selection was successfully achieved and led to changes in major histocompatibility complex (MHC) type distribution. Effects of MHC on the selected trait were subsequently assessed in the selected lines and in a F2. The low line was more susceptible to Marek's disease than the control line, but the high line was not more resistant than the control line. MHC types showed also significant effects on resistance to Marek's disease. Both antibody response and resistance to Marek's disease showed high heritabilities and effects of MHC but it could not be established whether disease resistance may be improved by selection for antibody response only.
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Marie-Hélène

STELLINGEN

-1- Both antibody response to sheep red blood cells (SRBC) and resistance to Marek's disease in chickens can be improved by direct selection for the specific characteristic features or selection for the favourable MHC alleles.

This thesis.

-2- It could not be proved that resistance to Marek's disease in chickens may be efficiently increased by improving antibody response to SRBC only.

This thesis.

-3- Heritability of resistance to a disease is not by definition low, as often stated.

This thesis.

-4- Improving genetic disease resistance in domestic animals, by means of e.g. selection, is often justified by ethical reasons. The same experiment in humans, however, would be, also for ethical reasons, forbidden.

-5- Immunogenetics as any multidisciplinary science is a combination of different sciences. Such integration should also exist among scientists.

-6- Spending time on candidate genes may be more disruptive than trying to find anonymous QTL.

M. Dentine, XXIII International Conference on Animal Genetics, Interlaken, Switzerland, August 5th 1992.

-7- In agricultural education, "Poultry" is always presented at a secondary level, as compared to e.g., dairy Cattle. As this tendency is still noticeable in research, efforts should be made to eliminate this established hierarchy.

-8- If something goes wrong in the course of a PhD (AIO) project, it is not always the fault of the "others".

-9- It took maybe a million of years to create this world. But because we were not present then and, therefore, we may never be able to explain every phenomenon, we have already spent centuries with research trying to compensate this frustration. Reaching this understanding regards somehow the research of ourselves too.

-10- It is not the task of Science to tell us what will be the man of tomorrow, but it is the responsibility and the privilege of the man of today to create the Science of tomorrow.

-11- Curiosity may not be necessary to do research, but it certainly helps by making research a natural continuous activity and by satisfying your desires. However, methodology and perseverance are, in addition, still required.

-12 - Genetic variability in humans is a precious element, fulfilling the need of every one of being recognized as unique by being different from the others; intolerance and eugenism are the denying that this difference can be source of richness.

M.-H. Pinard
Selection for immunoresponsiveness in chickens: effects of the major histocompatibility complex and resistance to Marek's disease.
Wageningen, 29 september 1992.

"C'est la Science qui nous exerce à mieux poser nos questions, donc à plus "être"; car être, n'est-ce pas d'abord s'interroger ?"

Albert Jacquard
(Au péril de la Science)

CONTENTS

GENERAL INTRODUCTION

GENERAL INTRODUCTION

RELEVANCE OF BREEDING FOR DISEASE RESISTANCE

Selection of livestock species has always been primarily limited to production traits. More recently, economic interest of improving reproduction performances has been considered; ways of improvement may involve direct selection or crossing with breeds having better reproduction performances as experimented in pigs. Likewise, when production performances of a breed have been successfully brought to a higher level, additional economic profit might by obtained by improving genetic disease resistance.

Indeed, economic loss from disease may arise from reduced production, loss of animals implying eventually reduced genetic gain, veterinary and medication costs etc... On the contrary, genetic disease resistance is inherited over generations, may even enhance vaccination efficiency in case of infectious diseases (Gavora, 1990) and therefore could be regarded as a preventive measure together with sanitary precaution and zootechnical measures. Moreover, not all infectious diseases can be prevented by vaccination. Also, several studies showed positive correlation between some production trait and disease trait, such as body or egg weight and mortality to Marek's disease in chicken (Gavora, 1990) or milk yield and occurrence of mastitis in dairy cattle (Shook, 1989). Therefore, these results predict an increase of disease susceptibility in the long term if selection pressure is applied only on particular production traits. Of course, selection for disease resistance is also time consuming and the final gain results from a complex balance between cost and profit. Research on ways of improving disease resistance is therefore very crucial in order to obtain efficient and cost limited selection methods which may be then included in present breeding programs. Besides the economic profit of improving disease resistance, the possibility of reducing medication would be an attractive feature regarding both public health and ethical concern, product quality and animal welfare.

WAYS OF BREEDING FOR DISEASE RESISTANCE

Research on how to improve genetic disease resistance involve intuitively two major steps: how to identify genes affecting disease resistance and how to use them. Direct selection for improved disease resistance is the most straightforward method. It has been successfully applied for resistance to Marek's disease (Friars et al., 1972; Gavora et al., 1986) or to Newcastle disease (Gordon et al., 1971) in chicken, and to *Brucella abortus* in cattle (Templeton et al., 1990) for example. However, direct selection has the major disadvantage to necessitate expensive extra progeny testing in animals and challenge facilities, and an increased generation interval. Therefore, methods consisting in selecting for a correlated non pathological trait or for genes having a positive effect on the disease trait will be regarded as attractive alternatives to direct selection. But to be of practical interest, the marker trait should be highly correlated with the disease trait, showing great genetic variability, and both marker trait and marker genes should be easily measured or identified, and preferably at an early age (Shook, 1989; Gavora, 1990).

In this study, immune traits and major histocompatibility complex (MHC) genes are prime candidates (e.g., Van der Zijpp, 1983; Warner et al., 1987; Lamont, 1989). Selecting for antibody response to a multideterminant antigen such as sheep red blood cells (SRBC) may improve a broad immune response (Van der Zijpp, 1983). Likewise, the MHC is a highly polymorphic group of genes which has been found in all vertebrates and shown to be involved primarily in immune response and disease resistance phenomena but also in reproduction and production (Warner et al., 1987; Bacon, 1987; Van der Zijpp and Egberts, 1989). Because of its linkage with the B blood group, the chicken MHC has been called the B complex and comprises three classes of genes encoding cell surface glycoproteins. The different classes correspond to different molecules, tissue distributions and functions. Identification of MHC types in individuals may be performed using serology, biochemistry or molecular techniques (reviewed by Guillemot and Auffray, 1989; Lamont and Dietert, 1990).

Poultry is an ideal species to conduct large selection experiments and has always been a model of choice for immunogeneticists. Moreover, the chicken is present in all cultures and religions, is the major animal source of protein in most countries including third world countries (NRC, 1991) and is therefore a very important species to study

(Simonsen, 1980).

OBJECTIVES OF THE STUDY

The main objectives of this study were to analyze, in chickens, the genetic control of antibody response to sheep red blood cells (SRBC) and of resistance to Marek's disease a disease caused by a herpes virus (Calnek, 1986; Schat, 1987), with special emphasis on the effects of MHC genes. Moreover, the possibility to use enhanced antibody response and MHC genes to improve resistance to Marek's disease was assessed.

Chickens were selected for high and low antibody response to SRBC. In addition, a control line was kept by random mating. In chapter 1, the results of nine generation of selection are presented; heritability and realized heritability of antibody response are estimated. In chapter 2, MHC antigens present in the chicken lines are characterized using serology and biochemistry. Association between MHC and antibody response to SRBC are assessed in two ways: Firstly, possible effects of selection on distributions of MHC types in the selected lines were analyzed, using the ninth and tenth generations animals (Chapter 3). Secondly, in order to understand whether the different MHC types distribution between the lines may be due to selection for antibody response, effects of MHC types on antibody response are estimated in the high, control and low lines (Chapter 3) and in a F2 which displays a random background (Chapter 4). Finally, the effects of selection for antibody response to SRBC and of MHC on resistance to Marek's disease are assessed by challenge of animals possessing different MHC types from the high, control and low lines, as well as from a F1. Results of this challenge are analyzed using a genetic epidemiological approach (Chapter 5).

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CHAPTER 1

DIVERGENT SELECTION FOR IMMUNE RESPONSIVENESS IN CHICKENS: ESTIMATION OF REALIZED HERITABILITY WITH AN ANIMAL MODEL.

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DIVERGENT SELECTION FOR IMMUNE RESPONSIVENESS IN CHICKENS: ESTIMATION OF REALIZED HERITABILITY WITH AN ANIMAL MODEL

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ABSTRACT

With the aim of improving general disease resistance, chickens were divergently selected for their antibody titers 5 d after immunization with sheep red blood cells for nine generations. Selected and control lines differed significantly for primary and secondary responses after three generations. Heritability of the antibody titer was estimated by REML fitting an animal model using a derivative-free algorithm. The heritability estimate using data on all lines simultaneously was .31. Realized heritability of the antibody titer in the selected lines was estimated by using either the phenotypic cumulative response as the deviation from the control line or the mean breeding values obtained with an animal model. Values from the two methods were consistent, giving a realized heritability of .21 and .25 in the high and low lines, respectively. The genetic trend was not linear and the response to selection tended to accelerate over generations.

Key Words: Chickens, Selection, Immune Response, Animal Model, Heritability

INTRODUCTION

Infectious diseases are responsible for major economic losses in livestock production. Although vaccines and medicines are widely used for prevention and treatment, they represent an expense and reduction in use may be required for ethical reasons. In comparison, genetic resistance may have several advantages: it is inherited and can enhance the response to vaccines (Gavora and Spencer, 1979; Gavora et al., 1990). Genetic resistance may be improved indirectly by selecting for a broad immune response. For example, chickens selected for high antibody response to sheep red blood cells (SRBC) showed higher resistance to some infectious diseases (e.g., Marek's and Newcastle diseases) but not to all of them (e.g., *E. Coli*) (Gross et al., 1980; Dunnington et al., 1986). By contrast, the lines of chickens selected for high antibody response to rabbit serum albumin or anaphylactic shock to BSA showed higher resistance to Marek's disease tumor cell line but no difference was observed in genetic resistance to Marek's virus (Yamamoto et al., 1991).

With the aim of improving general disease resistance, we selected chickens for their antibody response to a multideterminant, nonpathogenic antigen: sheep red blood cells (SRBC) (Van der Zijpp, 1983). Biozzi et al. (1979) successfully selected mice for antibody response to SRBC and showed the trait was onder polygenic control, which involved at least six loci.

The opportunity for selection on a trait depends on the heritability, which measures the amount of additive genetic variation in a trait (Falconer, 1989). Realized heritability has been traditionally estimated by using either a directional or divergent selection design (Hill, 1972a,b). Recent methodology and computational resources, however, allow the inclusion of data on all generations and relationships by using an Individual Animal Model (IAM), which has become the method of choice to analyze selection experiments (Sorensen and Kennedy, 1986).

The objective of this study was to present the results of nine generations of divergent selection for primary antibody response to SRBC, to characterize the ninth generation for correlated antibody titers, and, finally to compare the heritability estimated by the traditional method and by the use of an IAM.

MATERIALS AND METHODS

Selection Lines

Beginning in 1980, chickens were bidirectionally selected from an ISA Warren cross base population (generation G_0, n=614) for nine generations. The selection criterion was the total antibody titer 5 d after the primary i.m. immunization (P5TO) with 1 mL of 25% SRBC diluted in PBS at 37 d of age. The SRBC were pooled from five to seven unrelated Texel sheep. Selection was performed every year in nonoverlapping generations. Besides the high (H) and low (L) lines, a randombred control (C) line was kept. Every generation, there were approximately 300 chicks each in the H and L lines and 250 chicks in the C line, from which approximately 25 males and 50 females in H and L lines and approximately 40 males and 70 females in the C line were used to produce the next generation. In the H and L lines, males and females were selected on their individual titer values, whereas in the C line, random selection was used. Random mating of the parent was used; the only restriction was the exclusion of matings between full and(or) half-sibs. To measure secondary antibody response, all hens and the selected cocks were reimmunized according to the same procedure with 1 mL of 50% SRBC at 93 d of age.

Antibody Titers recorded

Total antibody titers and 2-Mercaptoethanol-resistant (immunoglobulin G [IgG]) titers were measured 0 (P0TO and P0Me) and 5 (P5TO and P5Me) d after primary immunization and 0 (S0TO and S0Me) and 5 (S5TO and S5Me) d after secondary immunization. Antibody titers measured against SRBC were expressed as the $\log_2$ of the reciprocal of the highest blood plasma dilution giving complete agglutination. Titrations were assessed in 96-well microtiter plates, using the same SRBC that was used for the immunizations. Assays were all performed the same day. Repeatibility of the titer determination was very high (Van der Zijpp and Leenstra, 1980). In the text, figures and tables, the term titer will refer to the $\log_2$-transformed values.

Housing and Vaccinations

Animals were reared in cages of 50 per 100 cm^2, with 10 chicks maximum per cage until 18 wk of age. After 18 wk of age, birds were housed in individual cages. Sexes were kept separate. All birds were vaccinated against Marek's disease at 0 d, infectious bronchitis at 1 and 126 d, infectious bursal disease at 15 d and Newcastle disease at 30 and 56 d of age. The light regimen was as used in commercial practice; feed and water were available ad libitum.

Statistical Analysis

Comparison between Lines.

The P0ME values were very low (99.8 % of the animals had a zero titer value) and were, therefore, not considered in the comparison. The other titers were checked for skewness and kurtosis with the UNIVARIATE procedure of SAS (1990). Differences between lines in G_9 were tested with a t-test for total titers and S5ME titers and with Fisher's test for P5ME titers because of their departure from normality. For the secondary response, only hens were considered because selection had already been performed in cocks. Tests were performed by SAS (1989).

Heritability of the Selected P5TO and Inbreeding.

Heritability was obtained by derivative-free restricted maximum likelihood (DFREML; Meyer, 1989), according to the following individual animal model (IAM):

$$Y_{jkl} = \mu + G_j + S_k + U_{jkl} + e_{jkl}$$

where

Y_{jkl} = the P5TO of the l^{th} chick,

μ = a constant,

G_j = the fixed effect of the j^{th} generation (0 to nine),

S_k = the fixed effect of the k^{th} sex of the chick,

U_{jkl} = the random additive genetic effect on the P5TO in the l^{th} chick and

e_{jkl} = a random error.

The fixed effect of generation accounted for environmental differences between generations, corresponding to different years. Generation X line interaction was found not significant and, therefore, was not included in the model. In G_0, all animals, including the parents for G_1, were considered. All relationships and data of the nine generations were used. Heritability was estimated across lines and within line. The average inbreeding level of the lines per generation was calculated from the additive genetic relationship matrix.

Realized Heritability of the PSTO.

Realized heritabilities (h^2_r) were estimated in H and L lines separately by regression of the cumulative selection responses (method 1) or the means of the estimated breeding values (method 2) on the cumulative selection differentials over generations.

Every generation, the selection differential was calculated as the mean of the selection differential of males and females where the number of progeny per parent was accounted for in calculating the selection differential. A generation X line interaction was found to be absent. The correction for environmental effect in method 1 was, therefore, performed as suggested by Hill (1972a,b): selection response was calculated from the difference between phenotypic means, expressed as deviation of selected lines from the C line, of two subsequent generations; thus, the cumulative response at a given generation was simply the phenotypic mean of a generation of the selected line deviated from the phenotypic mean of the C line, i.e., P_H-P_C and P_L-P_C for the H and L line, respectively.

In method 2, estimated breeding values (EBV) were estimated using the PEST program (Groeneveld, 1990; Groeneveld and Kovak, 1990) by applying the IAM described above on the whole population, assuming a heritability value (.31), which was estimated by DFREML previously from data across lines. Estimated breeding values were averaged per line and generation.

In both methods, h^2_r was estimated by forcing the regression through the origin, because the control and selected lines came from one unselected base population G_0 (Hill, 1972b).

RESULTS

Selected Antibody Titer (P5TO)

Generation means of the H, C and L line are given in Table 1. The H and L lines responded to selection for nine generations and differed ($P < .01$) from G_3 onward. In G_9, differences in mean P5TO between the selected lines and the C line reached a titer of 4.3 but P5TO distributions within lines were still overlapping due to large phenotypic variation (Figure 1). In all lines, females generated higher ($P < .01$) antibody titers than males (Table 2).

FIGURE 1. Distribution of the total antibody titers five d after primary immunization (P5TO) against sheep red blood cells in the low, control and high lines. The antibody titer refers to the $\log_2$-transformed value.

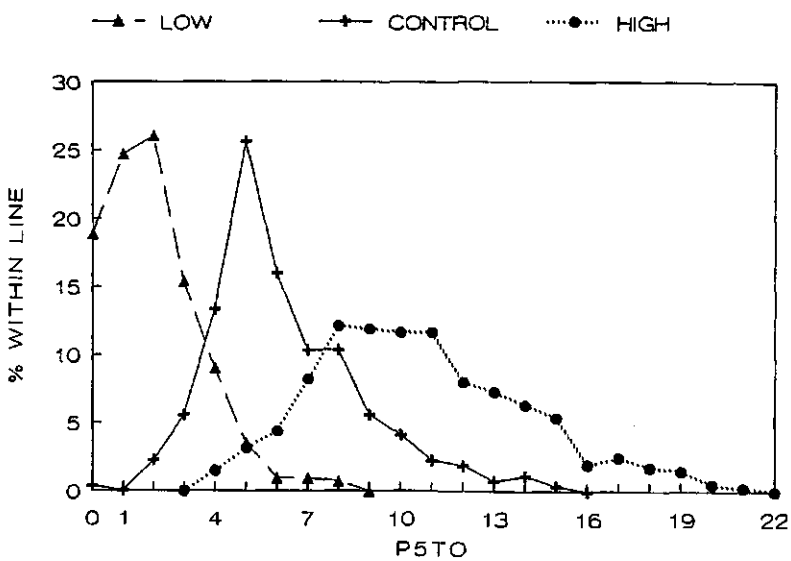


TABLE 1. Mean values (and their SD) of the total antibody titer against sheep red blood cells 5 d after primary immunization (P5TO) per line and generation[a]

	Line			
G[b]	High	Control	Low	n[c]
0	4.73 (1.05)	4.73 (1.05)	4.73 (1.05)	614
1	5.98 (2.39)	5.79 (2.01)	5.18 (2.02)	752
2	6.24 (1.84)	5.79 (1.52)	5.24 (1.79)	735
3	5.57 (1.86)	5.27 (1.73)	3.88 (1.62)	947
4	5.11 (2.27)	3.91 (2.04)	2.94 (1.93)	874
5	7.20 (1.75)	4.83 (1.61)	2.81 (1.53)	709
6	6.39 (1.55)	4.70 (1.43)	2.81 (1.56)	963
7	7.07 (1.65)	4.89 (1.83)	2.14 (1.59)	969
8	8.75 (2.10)	5.76 (2.08)	2.48 (1.50)	944
9	10.62 (3.38)	6.24 (2.46)	1.94 (1.57)	1110

[a]The antibody titer refers to the $\log_2$-transformed value
[b]Generation
[c]Total number of animals per generation

TABLE 2. Mean primary antibody titers (and their SD) against sheep red blood cells in the high (H), control (C) and low (L) lines of the ninth generation[a]

Line	Sex	n[b]	P0TO[c]	P5TO[d]	P5ME[e]
H	Hen	207	.55 (.83)	11.12 (3.37)^{s}	.19 (.50)
	Cock	204	.51 (.83)	10.11 (3.31)^{t}	.16 (.51)
C	Hen	136	.51 (.68)	6.60 (2.52)^{s}	.08 (.27)
	Cock	133	.60 (.88)	5.87 (2.34)^{t}	.08 (.28)
L	Hen	217	.34 (.64)	2.35 (1.66)^{s}	.00 (.00)
	Cock	213	.37 (.70)	1.53 (1.53)^{t}	.01 (.12)
H	ALL	412	.53 (.83)^{x}	10.62 (3.38)^{x}	.18 (.50)^{x}
C	ALL	269	.55 (.79)^{x}	6.24 (2.46)^{y}	.08 (.27)^{y}
L	ALL	430	.35 (.66)^{y}	1.94 (1.57)^{z}	.01 (.08)^{z}

[a]The antibody titers refer to the $\log_2$-transformed value
[b]Total number of animals
[c]Total titer 0 d after primary immunization
[d]Total and [e]IgG titer 5 d after primary immunization
$^{s\text{-}t}$:Different superscripts indicate differences ($P < .01$) between sexes within lines
$^{x\text{-}z}$:Different superscripts within columns indicate differences ($P < .01$) between whole lines

Correlated Titers

Mean primary and secondary titers in G_9 are presented in Tables 2 and 3, respectively. A low titer of P0TO was observed at day 0. Nevertheless, as was found by Kupper-Ron and Peleg (1983), these natural antibodies to SRBC-like antigens did not affect the following induced immune response to SRBC (data not shown). Except for the primary total titers and secondary IgG titers at day 0, the three lines differed ($P < .01$) from each other for other titers. Five days after immunization, secondary total titers were lower than primary total titers with the exception of the L line. However, in all lines, secondary IgG titers were very much higher ($P < .01$) than primary IgG titers 5 d after immunization.

Inbreeding of the Lines

After nine generations, average inbreeding level was low in the C line (3.6 %) and moderate in the selected lines (7.3 and 9.4 % in the H and L lines, respectively).

TABLE 3. Mean secondary titers (and their SD) against sheep red blood cells in females of the high (H), control (C) and low (L) lines of the ninth generation[a]

LINE	n[b]	S0TO[c]	S0ME[d]	S5TO[e]	S5ME[f]
H	203	1.83 (.83)^x	.01 (.09)	6.41 (1.18)^x	3.56 (1.05)^x
C	136	1.28 (.84)^y	.01 (.11)	5.13 (1.07)^y	2.56 (1.22)^y
L	217	.68 (.92)^z	.00 (.06)	3.83 (.99)^z	1.37 (1.08)^z

[a]The antibody titers refer to the $\log_2$-transformed value
[b]Total number of hens
[c]Total and [d]IgG titer 0 d after secondary immunization
[e]Total and [f]IgG titer 5 d after secondary immunization
$^{x\text{-}z}$:Different superscripts within each column indicate differences ($P < .01$) between lines

Heritability of the P5TO

Heritability of the selected trait (P5TO), estimated with an IAM using data on all lines over nine generations, was .31±.01. The estimate of the h^2 in the L line was higher (.36±.05) than in the H line (.29±.04) and the C line (.22±.04). To determine how the choice of the data set and the number of generations affected the estimate of h^2, the IAM was run from G_0 up to different end points (i.e., generations) and from different starting points until G_9, but still taking into account all relationships starting from G_0. An example using the H line is presented in Table 4. In the first case, the h^2 estimate was initially high when considering only G_0, then lower and finally increased to reach .29 by the G_9. By contrast, ignoring the base population and starting with later generations, h^2 increased to reach in G_9 the value of .49, which is the h^2 estimate within G_9 ignoring completely selection in all previous generations. As expected, the approximate SE of the estimate decreased for both approaches when taking more generations into account.

TABLE 4. Estimated heritabilities (h^2) of the total antibody titer five d after primary immunization (P5TO) against sheep red blood cells in the high line up to and from 0, 1, 3, 5, 7, 8 or 9 generations

	h^2	
G[a]	UP TO [b]	FROM[c]
0	.30 ± .08[d]	.29 ± .04
1	.19 ± .06	.34 ± .04
3	.16 ± .04	.37 ± .05
5	.16 ± .04	.45 ± .05
7	.17 ± .04	.48 ± .07
8	.22 ± .04	.49 ± .08
9	.29 ± .04	.49 ± .12

[a]Generation n
[b]h^2 estimated with the IAM using all relationships and data starting from G_0 and up to G_n.
[c]h^2 estimated with the IAM using all relationships in the nine generations and data starting from G_n and up to G_9.
[d]Approximated SE of the h^2 estimate

Realized Heritability of the P5TO

The selection differentials and cumulative selection differentials for the L line were constantly lower than those of the H line, the selection differential for the L line having an average value of 1.69 titer vs. 2.12 titer for the selection differential for the H line (Table 5). The selection pressure applied to produce G_6 was exceptionally low because of a small population size in G_5 (Table 1). Cumulative selection response (method 1) and means of EBV (method 2) for the H and L lines are shown per generation in Table 6 and plotted against the cumulative selection differential in Figure 2. Although the trends were very similar, method 2 showed a much smoother evolution than method 1 and gave an average yearly genetic gain of 0.48 and -0.44 titers for the H and L lines, respectively. No change in genetic level or inbreeding depression could be observed in the C line (Figure 2). Values of h^2_r estimated by method 1 and 2 were in total agreement and were higher in the L line (.25) than in the H line (.21). The SE of the regression coefficient were higher in method 1 (.03) than in method 2 (.01).

TABLE 5. Selection differentials and cumulative selection differentials for the total antibody titer five d after primary immununization (P5TO) against sheep red blood cells per generation[a]

G[b]	SD_H[c]	CSD_H[d]	SD_L[e]	CSD_L[f]
1	1.51	1.51	1.35	1.35
2	2.78	4.29	1.78	3.13
3	1.95	6.24	1.67	4.80
4	2.17	8.41	2.03	6.83
5	2.92	11.33	1.91	8.74
6	1.22	12.55	1.32	10.06
7	1.93	14.48	1.78	11.84
8	2.01	16.49	1.82	13.66
9	2.62	19.11	1.58	15.24

[a]The antibody titer refers to the $\log_2$-transformed value
[b]Generation n
[c]Selection differential and [d]Cumulative selection differential in the high line to produce G_n
[e]Selection differential and [f]Cumulative selection differential in the low line to produce G_n

Genetic trends were non-linear in all lines (Figure 2), response to selection accelerated. Individual regressions of mean difference in EBV between two subsequent generations on the selection differential applied were calculated (Figure 3). With the exception of G_6, "partial" h^2_r tended to increase over generations, from .14 to .40 and from .21 to .44 in the H and L lines respectively.

FIGURE 2. Cumulative selection response for the total antibody titers five d after primary immunization (P5TO) against sheep red blood cells, as the phenotypic deviation in P5TO of the high (H) and the low (L) lines from the control (C) line (P_H-P_C and P_L-P_C) and mean estimated breeding values for P5TO of the H (EBV_H) and the L (EBV_L) lines in relation to the cumulative selection differential (CSD); mean estimated breeding values for P5TO in the C line (EBV_C) per generation. The antibody titer refers to the $\log_2$-transformed value.

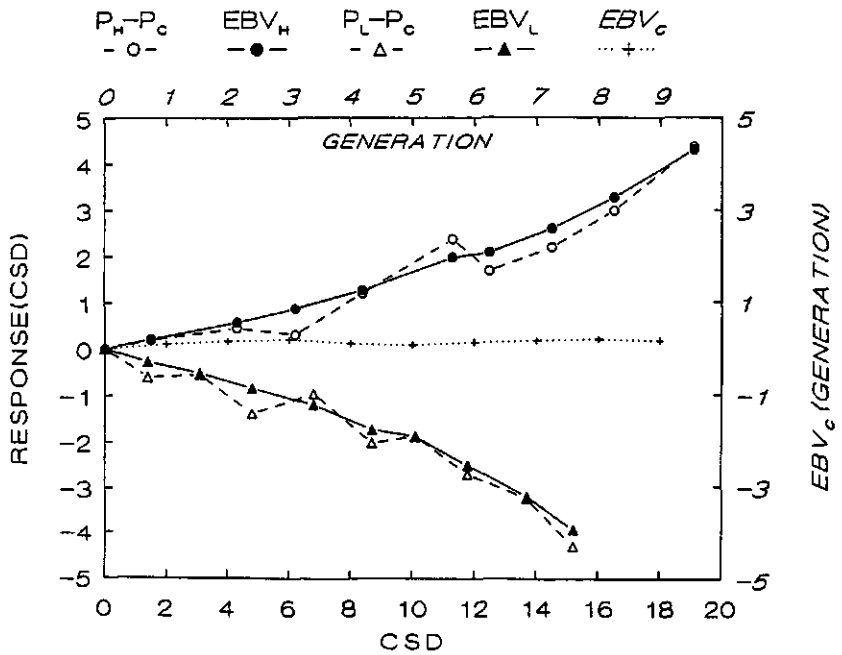
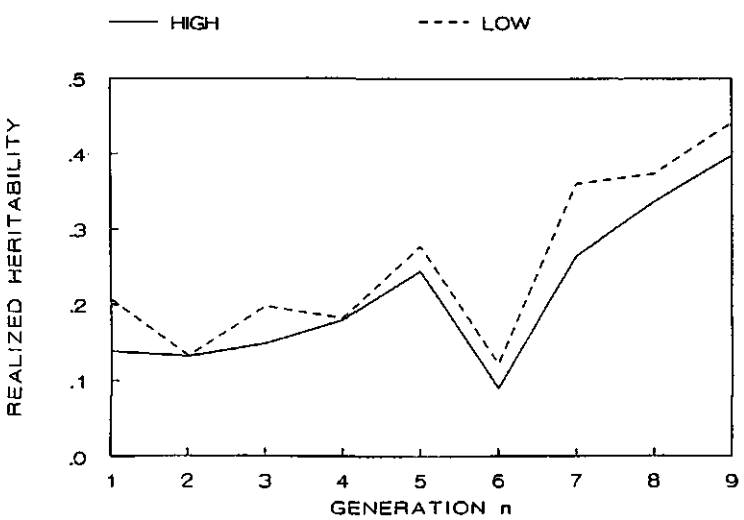


TABLE 6. Cumulative selection responses and mean estimated breeding values for the total antibody titer five d after primary immunization (P5TO) against sheep red blood cells per generation[a]

G^b	P_H-$P_C{}^c$	$EBV_H{}^d$	P_L-$P_C{}^e$	$EBV_L{}^f$
0	0	0	0	0
1	.19	.21	-.61	-.28
2	.45	.58	-.55	-.52
3	.30	.87	-1.39	-.85
4	1.20	1.26	-.97	-1.22
5	2.37	1.97	-2.02	-1.75
6	1.69	2.08	-1.89	-1.91
7	2.18	2.60	-2.75	-2.55
8	2.99	3.27	-3.28	-3.23
9	4.38	4.31	-4.30	-3.93

[a]The antibody titer refers to the $\log_2$-transformed value
[b]Generation
[c]Mean phenotypic value as deviated from the control and [d]Mean estimated breeding values in the high line
[e]Mean phenotypic value as deviated from the control and [f]Mean estimated breeding values in the low line

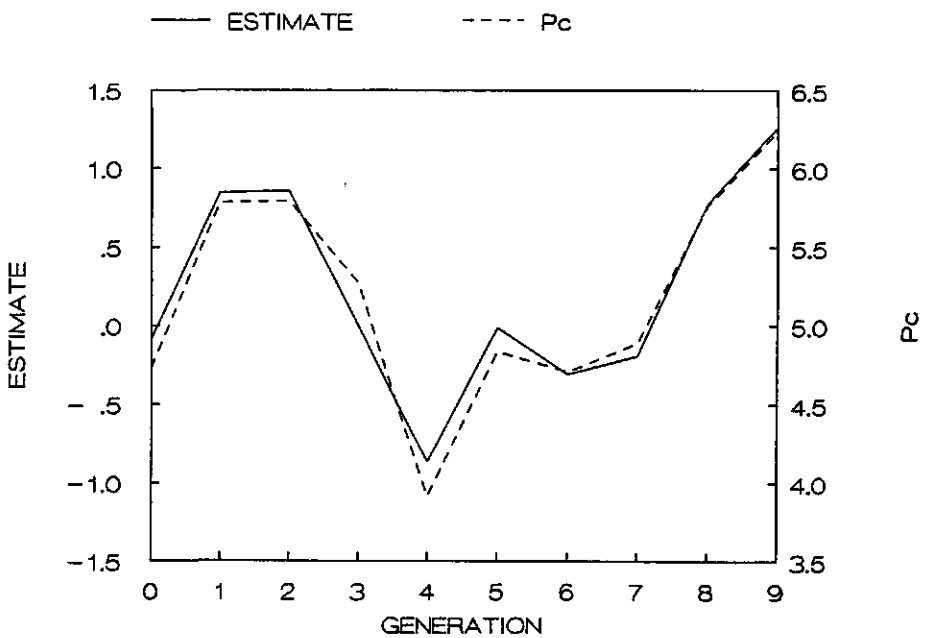
FIGURE 3. Partial realized heritabilities in the high and low lines between Generation n-1 and n using the regression of the means of EBV for antibody response to sheep red blood cells on the cumulative selection differentials (method 2).



Generation Effect on the P5TO

Generation effects, estimated from the IAM and the phenotypic evolution of the C line, gave similar trends (Figure 4). Differences between extremes reached 2.13 titers.

FIGURE 4. Estimate of the generation effect from the animal model (ESTIMATE) and phenotypic means in total antibody titers five d after primary immunization (P5TO) against sheep red blood cells of the control line (P_C) per generation.



DISCUSSION

Selection for high and low humoral immune response in the chicken was successfully achieved. The appearance of zero titer animals in the L line might be of concern. If the relative number of these animals continues to increase, it will no longer be possible to select downward identically on phenotypic value. Possible ways to select downwards among them are to establish a more sensitive test (i.e., an ELISA test) or to use the EBV of these individuals.

As also found by Martin et al. (1989) in a similar experiment, the three lines differed significantly for primary and secondary responses (Tables 2 and 3). Surprisingly, the secondary total titers were lower than the primary ones in the H line. This result can be interpreted as a negative relationship in the H line between the dose used for the primary response and the level of secondary antibody response (Kreukniet and Van der Zijpp, 1990), which might reduce the effectiveness of vaccination in this line. Moreover, lower secondary response may be regarded as a disadvantage in terms of immune memory and disease resistance.

A higher response of hens than cocks to SRBC and to a variety of other antigens have been reported by Siegel and Gross (1980) and Leitner et al. (1989). This dimorphism might be due to sex hormone effects on the thymus and the immune cells as in mammals (Grossman, 1984; Ansar Ahmed and Talal, 1990).

The generation effect was found to be responsible for a large variation in mean titer. Selection for immune response in goats showed a major environment effect (Eide et al. 1991). Here, sources for generation effect included differences in environment, handling and food intake although these factors were always held as consistent as possible. Even in a selection experiment with laboratory animals such as mice (Biozzi et al. 1979) major differences between years were observed.

To estimate the response to selection free from environmental trends, we used either the deviation from the control line or the fixed generation effect in the IAM. Both methods gave the same estimate of realized heritability. However, information from more generations was used when using the mean EBV and the genetic trend obtained was thus smoother, as found by Blair and Pollak (1984).

Values of EBV, used in method 2 to estimate realized heritabilities, depended on the input value of the heritability. As shown by Blair and Pollak (1984), the magnitude of mean EBVs increased with the input heritability. However, using heritability values from .20 to .40 would not have changed the estimate of realized heritability in this study.

Values of realized heritabilities were very close to those found in chickens by Martin et al. (1990) (.25 and .23 in the H and L lines, respectively, between the 10th and the 14th generations). Similar results were found in Guinea pigs by Ibañez et al. (1980) (.18 by interline divergence of eight generations) or in mice by Biozzi et al. (1979) (.21 by interline divergence of 13 generations having reached the selection limit). In the last two experiments, asymmetry in the response was reported and was interpreted in the latter case as an incomplete dominance of higher responsiveness over lower responsiveness. Here, we found higher (realized) heritabilities in the L line than in the H line, selection for a lower response appeared to be easier. Asymmetric response may be due here to - a base population not at an average phenotypic level, neither at a symmetrical gene frequency especially if some genes have a large effect; - a scale effect at different levels of the estimation. However, the question remains how genetically different the high responders differ now from the low ones, whether two different biological traits controlled by different genes were established. Or, as suggested by Cohen et al. (1985), "is low responsiveness an entity or only a deficiency ?"

Discrepancies between realized heritabilities and heritability estimates have been reported in many experiments and can be due to accumulation of inbreeding, gene frequency changes or mutations (James, 1990) but may not always be significant. Indeed, the standard errors of the regression underestimated the true standard error of the realized heritability because of sampling error in the generation mean estimates, especially when the difference between two lines was considered (Hill, 1972b). Moreover, the realized heritability estimated as a linear regression, could only be an approximation because the response was not linear.

Evolution of "partial" realized heritabilities (Figure 3) and the heritability estimated with the IAM by starting from the base population and taking increasing numbers of generations (Table 4) can be analyzed jointly. The decline of heritability in the early phase may be interpreted as a so-called "Bulmer effect" (Bulmer, 1971): selection leads to a reduction of additive genetic variance mainly due to the generation of linkage

disequilibrium. Therefore, a reduction of response after the first generation could be expected so that the realized heritability in early generations underestimated the true heritability (Falconer, 1989). Further variance can be progressively recreated by gene segregation and crossing overs, but only from the offspring of the second generation onwards until an equilibrium is reached (Robertson, 1977). But here, partial realized heritabilities were still increasing and the response to selection accelerating. Likewise, because gametic disequilibrium was not accounted for, a reduction of heritability estimate could be expected when ignoring previous selection, as found in a simulation study by Van der Werf and De Boer (1991). But here, omitting data from early generations (Table 4), all relationships still taken into account, led to an unexpected increase of heritability.

At the same time, in contrast to the observations in the selected mice of Biozzi et al. (1979), phenotypic variance of the P5TO was constant over generations. The constant variance in a similar selection of guinea pigs for SRBC has been interpreted as an increased sensitivity to environment (Ibañez et al., 1980), which can indeed become effective as the proportion of more sensitive homozygote animals increases under selection. Moreover, expected reduced genetic variance may be regained and even overcome by mutations (Hill and Keightley, 1988) and the various rearrangement of the genetic material (Frankham, 1990). These factors, however, are not likely to explain the continuous increase in heritability. It will be very interesting to see if this tendency remains in future generations. Finally, although we performed the analysis under the major assumption of a quantitative trait, i.e. equally affected by many individual loci, we may now search for genes directly involved in or as markers of the immune response.

To evaluate the consequences of an optimum selection scheme, effects of improving antibody response on other immune traits and association with production traits need to be assessed. As found by Martin et al. (1990), high-line hens had lower juvenile bodyweights than did low-line hens (Nieuwland et al., 1989) but Martin et al. (1990) observed the reverse at 38 wk of age; moreover, Kim et al. (1987) found a significant positive relationship between antibody response to SRBC and bodyweight from 2 to 6 wk of age in low responders to glutamic acid-alanine-tyrosine (GAT), but no correlation was observed in the high-responder group. Even if the altered level of humoral response is not restricted to SRBC, defensive functions include also phagocytosis and cell mediated immunity. In mice, improving humoral response did not modify T-cell-mediated immunity

but decreased macrophage catabolic activity, leading to an increased resistance to extracellulair pathogen infections but to an increased sensitivity in case of intracellulair pathogen infections (Biozzi et al., 1984). In our lines, preliminary studies did not show any effect on cell mediated immunity or macrophage activity (Van der Zijpp and Nieuwland, 1986; Van der Zijpp et al., 1988b; 1989) and the high line showed higher resistance to Marek's disease than the low line (Van der Zijpp et al., 1988a). Further immunological characterization and disease challenges need to be performed.

IMPLICATIONS

Selection for high and low antibody response was successfully performed in chickens for nine generations. Because of major between-year variation, a control line was necessary for correctly estimating the response to selection when using phenotypic response. Even if a mixed animal model is used, a control line should be included to account for genetic drift. In addition, the control line is important as a reference for the evaluation of the possible effects of selection on disease resistance and production traits.

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CHAPTER 2

BIOCHEMICAL AND SEROLOGICAL IDENTIFICATION OF MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS IN OUTBRED CHICKENS.

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BIOCHEMICAL AND SEROLOGICAL IDENTIFICATION OF MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS IN OUTBRED CHICKENS.

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ABSTRACT

Serology and biochemistry were used to identify major histocompatibility complex (MHC) types in chickens lines selected for high and low antibody response to sheep red blood cells. Serological typing was performed by direct haemagglutination, using antisera obtained by erythrocyte alloimmunization within the lines. Four serotypes were identified, called preliminarily B^{14}, B^{19}, B^{21} and B^{24}. Subsequently, these types were characterized for their B-G and B-F products by biochemical analysis, using SDS-PAGE and one-dimensional IEF respectively. The B^{14}, B^{19} and B^{21} serotypes displayed each characteristic banding patterns for both B-G and B-F. No additional types or subtypes were identified by biochemistry within these serotypes. The B^{24} serotype however could be subtyped into three different haplotypes with specific banding patterns for B-G and B-F. None of the haplotypes in the selection lines were identical for both B-G and B-F with the tested reference B haplotypes. Comparison of B-G alleles revealed similar but not identical B-G patterns for B^{14} and B-G^{14}, whereas B^{24C} (one of the B^{24} subtypes) and B-G^{23}, as well as B^{19} and B-G^{19} displayed indistinguishable patterns. For B-F, only B^{21} and B-F^{21} banding patterns were indistinguishable by IEF. All other B-F types differed from the reference types. The refined definition of MHC haplotypes of the selection lines will enable a more precise analysis of effect of MHC polymorphism on the immune response and on resistance to Marek's disease.

key words: chicken, MHC, serology, SDS-PAGE, IEF

INTRODUCTION

The avian major histocompatibility complex (MHC) was originally described as a three-locus complex (Pink et al. 1977) but is regarded now as a three-region complex, each region comprised of several genes encoding one of the three classes of molecules. The B-F and B-L gene products are, respectively, equivalent to the class I and class II gene products found in mammals (Guillemot & Auffray 1989). The B-G molecules, or class IV products, are highly polymorphic but have so far only been identified in the avian system. Until recently, B-G antigens were believed to be expressed only on erythrocytes (Salomonsen et al. 1987) and in experimental conditions they appeared to act as an adjuvant in immune responses (Hàla et al. 1981; Salomonsen et al. 1991a). New findings indicate that B-G molecules are also present on many other cells including thrombocytes, B and T lymphocytes (Salomonsen et al. 1991b). This finding will likely stimulate the search for the function of these molecules (Kaufman & Salomonsen 1992).

Typing for MHC can be performed by serology, biochemistry or restriction fragment length polymorphism (Guillemot & Auffray, 1989). Biochemical analyses are based on the polymorphism in molecular size (SDS-PAGE) or in net charge of the molecule (IEF). These techniques can be used independently or can be combined in a 2-Dimension analysis (Hepkema et al. 1991; Miller et al. 1984).

As in the case in other species, the MHC genes of chickens have been shown to be directly involved in immune responses (Lamont & Dietert 1990) and associated with resistance to several diseases (Bacon 1987). Therefore, in lines of chickens selected for high and low immune response to sheep red blood cells (van der Zijpp et al. 1988; Pinard et al. 1992), possible involvement of MHC genes needs to be investigated. A preliminary study showed differences in serologically defined MHC haplotypes between the selected lines (Pinard et al. 1990). Effects of MHC genes on the immune response and on resistance to infectious diseases are currently being investigated in our lines. Therefore, MHC types of the selected lines need to be well characterized and possible similarities with reference B-types need to be established so that our results can be compared with the results of other studies. Biochemistry was for this purpose the method of choice.

The goals of this study were to characterize the B-G and B-F products present in our selected lines, to assess whether the serological typing could be refined by the use of

biochemistry, and to relate our haplotypes to known White Leghorn reference types.

MATERIALS AND METHODS

Animals

Selected lines of chickens

Three lines of chickens, which originated from an ISA Warren cross were used: two lines selected for high (H) and low (L) humoral immune responses to SRBC for nine generations and a randombred control (C) line (van der Zijpp et al. 1988).

For the comparison of serological and biochemical typing of B-G products, all the seventh generation animals that were parents of the eighth generation (n=268), seven families comprised of 40 animals of the eighth generation with their parents, and 22 animals of the ninth generation were used.

For the biochemical typing of B-F products, 24 animals from different families and lines of the eighth and ninth generations were used.

Reference chickens

Reference material was obtained from the following sources: The Basel/Copenhagen chicken lines including reference lines for B-complex types B^{14}, B^{19} and B^{21}; and the Northern Illinois University (NIU) (Briles et al. 1982) reference flock comprised of ten families of 45 heterozygous animals segregating the following B-complex types: B^{2}, B^{3}, B^{6}, B^{8}, B^{11}, B^{12}, B^{14}, B^{15}, B^{17}, B^{18}, B^{19}, B^{R5} (R5=B-G^{19} B-F^{21}), B^{21} and B^{R6} (R6=B-G^{23} B-F^{21}).

MHC typing

Serological typing

Blood samples were taken from the wing vein and erythrocytes were washed three times in phosphate buffer saline (PBS). Typing for the MHC antigens expressed on the

erythrocyte surface was performed by direct haemagglutination in 96-well round-bottomed plates (Greiner, Alphen, The Netherlands). Twenty five µl of 2% packed red cells in PBS and 25µl of antisera were used per well. Before scoring, plates were shaken three times for one, two and three minutes followed by five, 15 and 45 minutes rest respectively.

Initially, cluster analysis was performed with cross reactive alloantisera produced in other populations (Briles & Briles 1982). Later, we developed our line specific alloantisera, which have been exclusively used in this analysis. Alloantisera were raised within families by three to five weekly intramuscular alloimmunizations of 2.5ml packed erythrocytes. The antisera were screened in serial dilution against animals from the population and were made specific, if necessary, by appropriate absorptions.

Biochemical typing

Biochemical typing was performed for both B-G (MHC class IV) and B-F (MHC class I) proteins, as described by Hepkema et al. 1991.

For B-G characterization, erythrocyte lysates were separated by SDS-PAGE (12% acrylamide), transferred to nitrocellulose and visualized with monoclonal antibody (mAb) 18-6G2 and a peroxidase conjugated second antibody. Relative mass (M_r) was estimated by comparison with prestained M_r markers.

For characterization of B-F molecules, peripheral blood lymphocytes (PBL) were radiolabeled overnight with 250µCi ^{35}S-methionine. The B-F products were then immunoprecipitated using mAb recognizing monomorphic determinants of the B-F heavy chain (F21-2) or beta-2-microglobulin (F21-21). After extensive neuramidase treatment, the immunoprecipitates were analyzed by one-dimensional isoelectric focusing (IEF).

All gels were run under reduced conditions. Banding patterns were used for the assignment of B-G and B-F alleles. Monoclonal antibodies were kindly provided by K. Skjødt (Department of Microbiology, Odense University, Denmark) and J. Salomonsen (Basel Institute for Immunology, Basel, Switzerland).

RESULTS

Serotype identification

Initially, three different B-types, B^{114}, B^{121} and B^{124} were identified by serology. However, there were also seroblank animals. Parallel SDS-PAGE analysis of B-G products in family material resulted in the identification, among seroblank animals, of a fourth type, called B^{119}, against which antisera were raised. Names for the serotypes were chosen because of serological cross reactivity with reference types. However, the prefix 1 was added to indicate that it was a preliminary designation. The four serotypes accounted for about 95% of the haplotypes, 5% remaining seroblank.

Biochemical analysis of B-G types and comparison with reference types

Figure 1 shows the characteristic SDS-PAGE patterns of B-G types associated with serologically defined MHC types and provides a comparison with reference B-G types: The B^{114} serotype (lane 1) was characterized by two bands: an intense higher band, also seen in the Basel reference B^{14} (lane 2), and a faint lower band, which could only be visualized in some B^{114} animals. The difference in intensity for the higher band between the B^{114} and the B^{14} was reproducible for the same animals and for different animals. Therefore, B-G alleles of B^{114} and B^{14} showed similarities but were not identical by this analysis. Comparison with the NIU reference B^{14} animal (lane 5) was difficult since no homozygous B^{14} animal was available and the B^{14} band was only slightly heavier than the middle band of the B^{19} haplotype. The B^{119} serotype (lane 3) and both the Basel and NIU reference animals for B^{19} (lanes 4 and 5, respectively) displayed indistinguishable patterns consisting of three bands. This indicated biochemical similarity or possible identity between the B^{119} and B^{19} B-G alleles. On the contrary, the B^{121} haplotype (lane 6) and the Basel reference B^{21} (lane 7) had completely different patterns, indicating different B-G alleles.

Serotype B^{124} displayed at least three distinct B-G banding patterns, called B^{124A} (lanes 8 and 9), B^{124B} (lane 9) and B^{124C} (lane 10 and 11). Biochemical typing of 268 animals of the seventh generation resulted in the identification of only four B^{124C}

haplotypes in three animals from the low line and one B^{124C} haplotype in each of the other two lines, i.e. only 3.8% of all B^{124} haplotypes were of B^{124C} type. On the other hand, the B^{124A} and B^{124B} patterns were associated with large numbers of B^{124} animals. The anti B^{124} serum which reacted strongly with all B^{124} subtypes was raised against a donor carrying the B^{124C} subtype. Sera raised against the other subtypes were much less specific and were much weaker.

The B^{124C} subtype, characterized by two bands, and R6 (=B-G^{23} B-F^{21}) displayed indistinguishable patterns, indicating that B^{124C} and R6 might share, at least partly the same B-G alleles. No biochemical similarity was found between the other B^{124} types and any of the B-G types carried by the available reference animals.

An example of the segregation of the B^{119}, B^{121} and B^{124C} types in a family of the high line is shown in Figure 2.

It was not possible to obtain an accurate MHC typing of our lines by using B-defined antisera, which originated from White Leghorn animals. Consequently, to confirm serologically the similarities and differences established at the biochemical level, we tested our sera for the B^{114}, B^{119}, B^{121} and B^{124} types on the NIU reference flock. Results were in agreement with the findings of the biochemical analysis (Table 1).

TABLE 1. Reactivity of the B^{114}, B^{119}, B^{121} and B^{124} antisera with erythrocyte alloantigens associated with 13 B-haplotypes of the NIU flock.

	B-haplotypes[a]													
antis[b]	B^{2}	B^{3}	B^{6}	B^{8}	B^{11}	B^{12}	B^{14}	B^{15}	B^{17}	B^{18}	B^{19}	B^{R5}	B^{21}	B^{R6}
B^{114}	.[c]	.	.	.	.	.	+[d]	.	.	.	.	.	.	.
B^{119}	.	.	.	.	.	.	.	.	.	.	+	+	.	.
B^{121}	.	.	.	.	.	.	.	.	.	.	.	.	.	.
B^{124}	.	.	.	.	.	.	.	.	.	.	.	.	.	+

[a] R5=B-G^{19} B-F^{21} and R6=B-G^{23} B-F^{21}

[b] antiserum specificity

[c] . indicates no specific reaction

[d] + indicates specific reaction of the antiserum (e.g. B^{114}) on the NIU animals carrying the B-haplotype (e.g. B^{14}) and disappearance of the reaction specific to this antiserum (e.g. B^{114}) on the animals of the selected lines after absorption with the red blood cells of these NIU animals.

FIGURE 1. Immunoblots of lysates of erythrocytes separated on 12% SDS-PAGE gels and visualized with monoclonal antibody 18-6G2 against B-G antigens. Serotypes are indicated above the lanes. Serotypes comprised of one type represent homozygous animals for this type. M_r is in kD.

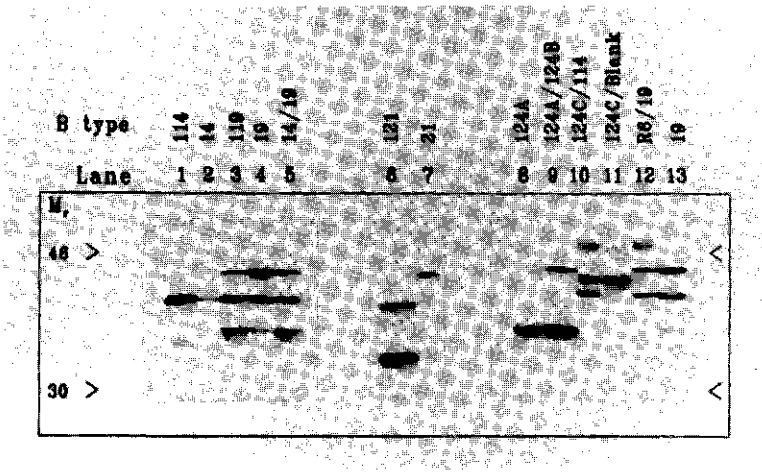
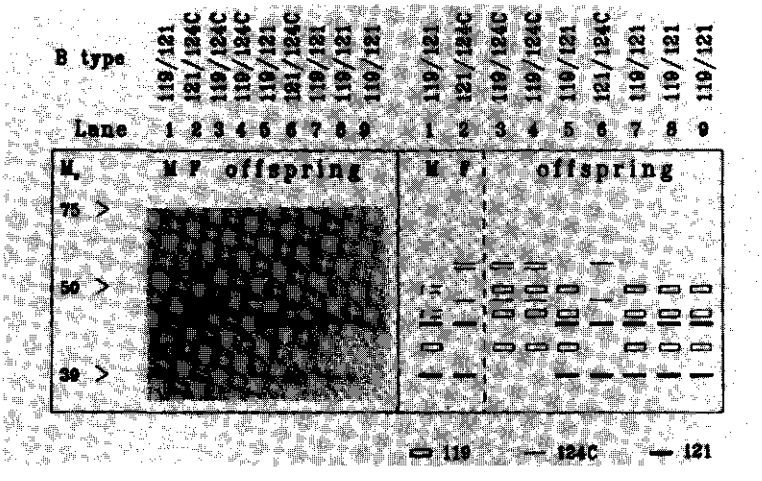


FIGURE 2. B-G antigens of a family from the high line separated on 12% SDS-PAGE gels and visualized with monoclonal antibody 18-6G2. The family is comprised of two parents (M: mother and F: father) from the seventh generation and offspring from the eighth generation. The left half of the figure shows the immunoblot while the right half shows the corresponding schematic drawing. The dotted boxes represent faint bands of the B^{119} haplotype.



Comparison between serological and biochemical typing for B-G types

Typing by serology and biochemistry of the 268 animals from the seventh generation was in agreement for, overall, 80.5% of the haplotypes studied; however the level of agreement varied for the different haplotypes (Table 2). Typing discrepancies involving established serotypes and SDS-PAGE patterns did not occur. However, 11.5% of the sero-haplotypes were associated with unidentified SDS-PAGE patterns due to additional or missing bands, and 7.5% of haplotypes did not display any SDS-PAGE pattern.

TABLE 2. Comparison of MHC typing by serology and SDS-PAGE of 268 animals of the seventh generation.

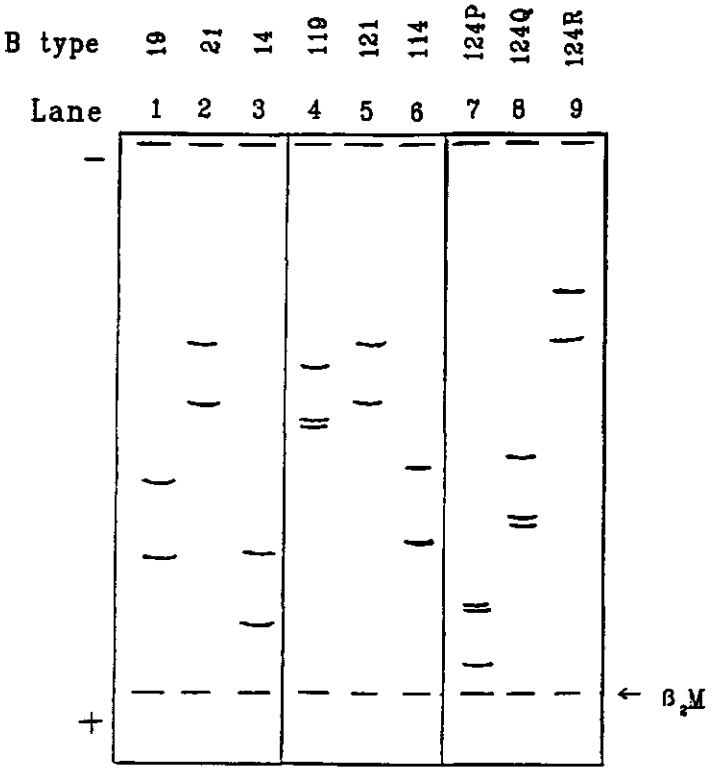
	serology					
SDS-PAGE	B^{114}	B^{119}	B^{121}	B^{124}	Blank	Total[c]
B^{114}	89[a](75.4[b])					89
B^{119}		83(89.2)				83
B^{121}			123(84.2)			123
B^{124}				130(77.8)		132
Unknown	22(18.7)	6(6.5)	15(10.3)	19(11.4)		62
Blank[d]	7(5.9)	4(4.3)	8(5.5)	18(10.8)	12(100)	49
Total[e]	118	93	146	167	12	536

[a] number of haplotypes identified by SDS-PAGE and by serology
[b] Percent of the haplotypes with a given serological allele associated with a particular SDS-PAGE type
[c] number of haplotypes with a given allele identified by SDS-PAGE
[d] B-G haplotype not recognized by the 18-6G2 mAb
[e] number of haplotypes with a given allele identified by serology

Biochemical analysis of B-F types

For the B^{14} and B^{21} serotypes, four homozygous animals and one heterozygous animal per serotype (nine haplotypes per serotype) were analyzed by IEF. For the B^{19} serotype, four homozygous animals (eight haplotypes) were analyzed by IEF. For the B^{24} type, 18 haplotypes carried by 7 homozygous and 4 heterozygous animals were studied. Comparison with reference animals was performed and results are represented by an interpretative drawing in Figure 3: B^{14} (lane 6), B^{19} (lane4) and B^{21} (lane 5) each displayed a characteristic B-F banding pattern whereas B^{24} B-F patterns were, as for B-G, split into three subtypes called B^{24P} (lane 7), B^{24Q} (lane 8) and B^{24R} (lane 9). The B^{14} and B^{19} types had IEF patterns that where clearly different from those of the reference animals for B^{14} (lanes 3) and B^{19} (lane 1) respectively. However, the B^{21} type and the reference B^{21} type (lane 5) had indistinguishable IEF patterns. None of the B^{24} patterns showed any similarity with the available reference animals. In addition, the B^{14} (lane 6) showed some similarity with the reference B^{19} (lane 1), but repeated analysis showed slight differences in height between the two haplotypes. Most of the haplotypes displayed two bands, but the upper one appeared often weakly. For further analysis of the reference types, see Hepkema et al. (1991).

FIGURE 3. Interpretative drawing of one dimensional isoelectric focusing of B-F immunoprecipitates of radiolabelled PBL using the B-F specific monoclonal antibody F21-2. for IEF patterns of reference B-types see Hepkema et al (1991).



Haplotypes

All animals typed by IEF for B-F alleles were also typed by SDS-PAGE for B-G alleles and haplotypes were defined as the combination of B-G and B-F alleles.

All nine B^{114} and B^{121} haplotypes and all eight B^{119} haplotypes consisted of the same combination of B-G and B-F alleles. All 18 haplotypes serologically typed as B^{124} showed the same combination of B-G and B-F alleles: 13 haplotypes were combinations of B-G^{124A} and B-F^{124P}, one haplotype displayed B-G^{124B} with B-F^{124Q} and four haplotypes consisted of B-G^{124C} with B-F^{124R}. In addition, one serologically blank haplotype was also blank by SDS-PAGE but was identified as B-F^{119} by IEF. An other serologically blank (lane 11; Figure 1) appeared to be B-G^{Blank} and B-F^{121}.

In conclusion, with the exception of serological blanks, no evidence for recombination between B-G and B-F alleles was identified in any of the haplotype studied here.

The B-G and B-F alleles identified in this study, and similarities or differences with haplotypes carried by reference animals are summarized in Table 3.

TABLE 3. Haplotype characteristics in comparison with reference types.

B-serotype	B-G SDS-PAGE	B-G Ref.	B-F IEF	B-F Ref.
114	114	≡[a] 14	114	#[b] 14
119	119	=[c] 19	119	# 19
121	121	# 21	121	= 21
124	124A	# R6	124P	# R6[d]
	124B	# R6	124Q	# R6
	124C	≡ R6	124R	# R6

[a] ≡ similar patterns
[b] # different patterns
[c] = indistinguishable patterns
[d] R6 = B-G^{23} B-F^{21}

DISCUSSION

The B-G molecules are highly polymorphic and 2-Dimensional gel electrophoresis has revealed that they have a complex structure (Miller et al. 1984). There is now evidence that the molecular weight polymorphism of B-G molecules, as shown here by SDS-PAGE, is related to a variation in length of the cytoplasmic part of the protein (Kaufman et al. 1989, 1990). On the contrary, serological typing detects mainly extracellular polymorphic determinants. Nevertheless, all serotypes displayed specific banding patterns on SDS-PAGE.

Different classification by SDS-PAGE and serology can be caused by polymorphism of antigenic determinants recognized by alloantisera, which may have not changed the molecular weight, resulting in different SDS-PAGE banding patterns. Most of the discrepancies between serotyping and biochemical typing were in fact due to missing or additional bands in the SDS-PAGE pattern or even total blanks. Variation in intensity of bands or missing bands can be caused by variation in the quantity of antigen expressed, bad recognition of the antigen by the mAb or bad transfer from the gel to nitrocellulose.

The issue of the B^{124} subtypes is intriguing. The three B^{124} subtypes differed in terms of molecular weight but were recognized by the same antiserum. However, polyclonal antisera usually recognized more than one antigenic determinant. The strong supertypic B^{124} antiserum was successfully raised only when the B^{124C} cells were used as the immunogen. Therefore, we can hypothesize that the B^{124C} subtype cells carry antigenic determinants, which include some of those carried by either the B^{124A} and/or the B^{124B} subtype cells.

As is the case with humans (Neefjes et al. 1986) and with cattle (Joosten et al. 1988; Viuff et al. 1991), IEF proved to be a valuable technique for characterizing MHC class I antigens because different B-F haplotypes produce molecules differing in their net charge.

Analysis of B-G and B-F molecules by SDS-PAGE and IEF, resulted in the identification of multiple bands per haplotype, tending to indicate multiple B-G and B-F products. For class I MHC antigens, duplicated bands, separated from each other by approximately one charge unit, have been identified in all other species, and represent

most likely products of the same gene (Neefjes et al. 1986; Joosten et al. 1988; Guillemot et al. 1989). For B-G, it has been shown that more than one gene is expressed (Goto et al. 1988; Kaufman et al. 1989). Multiple B-G gene expression could be partly responsible for the variety of the B-G molecules detected. However, as may be true for the multiple B-F bands, posttranslational events could also contribute to the diversity (Miller et al. 1991; Kaufman & Salomonsen 1992).

In conclusion, B-G typing using SDS-PAGE proved to be a complementary technique to serology, although, as previously described by Hepkema et al. (1989, 1991), not all B-G products were detected by the 18-6G2 mAb. In this study, the SDS-PAGE typing was especially helpful for identifying new types after the majority of types were identified serologically. However, if no alloantisera are available, the first identification of different B-types can also be obtained by biochemistry by using, preferentially, segregating families. Moreover, whereas serology is still more suitable than biochemistry for large scale typing, prior typing by biochemistry enables the production of the most specific alloantisera from appropriate donor-recipient pairs. With the addition of IEF typing for B-F alleles, haplotypes present in our selected lines could be characterized for their B-G and B-F alleles. However, the occurrence of faint, missing, overlapping or extra bands sometimes made the interpretation difficult. Thus, we recommend again, if possible, to do the biochemical analysis using segregating families, in order to reduce the risk of mistyping. Finally, we found that for our population, which was neither inbred nor of White Leghorn origin, biochemistry enabled the most precise characterization of haplotypes and the comparison of B-G and B-F alleles with reference B-alleles.

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CHAPTER 3

DIVERGENT SELECTION FOR IMMUNE RESPONSIVENESS IN CHICKENS: DISTRIBUTION AND EFFECT OF MAJOR HISTOCOMPATIBILITY COMPLEX TYPES.

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DIVERGENT SELECTION FOR IMMUNE RESPONSIVENESS IN CHICKENS: DISTRIBUTION AND EFFECT OF MAJOR HISTOCOMPATIBILITY COMPLEX TYPES.

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ABSTRACT

Chickens were selected for ten generations for high and low antibody response to sheep red blood cells; in addition, a randombred control line was maintained. All animals (n = 1602) from the ninth and tenth generations were typed for major histocompatibility complex B-types. All identified types were present in the control line but the selected lines showed divergent distributions. The 121 B-haplotype was predominant in the high line in the form of 121-121 B-genotype, whereas the 114 B-haplotype was most frequent in the form of 114-114 and 114-124 B-genotypes in the low line. To explain these frequency changes, effects of B-genotypes on the selected trait were estimated, using a mixed animal model. The B-genotypes were responsible for a significant part of the variation of the trait within lines, but their effects differed between lines. These effects could be related partly to the changes in B-genotype distribution. Interference of the selected background in the estimation is discussed.

Key words: chicken, immune response, selection, animal model, major histocompatibility complex

INTRODUCTION

In recent years, there has been a growing interest in improving the genetic resistance of domestic species to infectious diseases. This improvement may be accomplished indirectly by selective breeding for immune responsiveness and/or for genes or marker genes for immune responsiveness and disease resistance (Warner et al, 1987). Moreover, advances in molecular technique have opened promising ways for directly introducing advantageous genes into animals by genetic engineering (Lamont, 1989).

Chickens have been successfully selected for high and low antibody response to sheep red blood cells (SRBC) (Van der Zijpp et al, 1988). Pinard et al (1992) have found a heritability estimate for the selected trait to be 0.31. However, even if the humoral response to SRBC is under polygenic control, some specific genes might play a major role, and the genes of the major histocompatibility complex (MHC) are prime candidates. The MHC genes encode highly polymorphic cell surface proteins that have been shown to play an important role in immune responsiveness and disease resistance in many species including chickens (Bacon, 1987; Gavora, 1990; Lamont and Dietert, 1990).

Estimation of MHC-type effects remains a delicate task, especially in the framework of selected outbred lines. Ignoring the relationships between individuals may, for example, often lead to a overestimation of the MHC effect (Mallard et al, 1991). The choice of the method to estimate single gene effects separately from the background genes is therefore crucial (Kennedy et al, 1992).

The objectives of this study were to look for possible changes in MHC haplotype and genotype frequencies in lines of chickens divergently selected for ten generations for antibody response to SRBC, and to estimate the MHC effects on the selected trait in order to understand the involvement of MHC in the regulation of the immune response.

MATERIALS AND METHODS

Selection lines

The selection experiment has been described in detail elsewhere (Van der Zijpp et al, 1988; Pinard et al, 1992). Briefly, chickens were bidirectionally selected from an ISA Warren cross base population for ten generations. The selection criterion was the total antibody (Ab) titer, five days postprimary immunization with 1 mL 25% sheep red blood cells (SRBC). In addition to the high (H) and low (L) lines, a randombred control (C) line was maintained. The numbers of animals in the H, C and L lines of the ninth and tenth generations are given in table I.

Typing for MHC haplotype

Major Histocompatibility Complex haplotypes were determined by direct haemagglutination, using alloantisera obtained from the lines. Four serotypes, provisionally called B^{114}, B^{119}, B^{121}, and B^{124} were identified in the tested animals (Pinard et al, 1991; Pinard and Hepkema, 1992). A MHC genotype was defined as the combination of two haplotypes. Serological typing was performed on the parents of the eighth generation, on all the females and the selected males of the eighth generation, and on all the animals of the ninth and tenth generation. Only the results of MHC typing in the ninth and tenth generations were used in the analysis. Segregation of the haplotypes was checked for consistency within families over generations, and inconsistent data were removed from the analysis.

Statistical analysis

Comparison of MHC type frequencies between the lines was performed by tests of chi-square.

Effects of MHC genotype on the Ab response were estimated within lines by PEST (Groeneveld, 1990; Groeneveld and Kovak, 1990) using the following mixed model:

$$Ab_{ijklm} = \mu + generation_i + sex_j + line_k + MHC_{kl} + U_{ijklm} + e_{ijklm}$$

Where:

Ab_{ijklm} = the Ab titer of the m^{th} chick,
μ = a constant,
$generation_i$ = the fixed effect of the i^{th} generation (9, 10),
sex_j = the fixed effect of the j^{th} sex of the chick,
$line_k$ = the fixed effect of the k^{th} line (H, C, L),
MHC_{kl} = the fixed effect of the l^{th} MHC genotype within the k^{th} line,
U_{ijklm} = the random additive genetic effect on the Ab titer in the m^{th} chick and
e_{ijklm} = a random error.

The fixed effect of generation accounted for environmental differences between generations nine and ten. The sex effect corrected for a higher Ab response to SRBC in females than in males. Relationships between individuals from the ten generations and Ab data of the ninth and tenth generations were used in this study. The mixed model was applied assuming a heritability of 0.31, as estimated previously (Pinard et al, 1992).

Differences between genotypes within lines were tested using the F test values as estimated by PEST. The overall effect of genotypes in a line was estimated by testing, jointly, n-1 independent differences, with n being the number of genotypes in the line.

Heterozygote superiority was estimated within line for each available combination of haplotypes by testing the difference between the heterozygote genotypes and the average of their homozygous counterparts. The overall heterozygote superiority in a line was estimated by testing the difference between these heterozygote genotypes and the average of their homozygous counterparts.

The effect of haplotype i was estimated within line by testing the difference between genotype combinations comprised of the haplotype i and their counterparts comprised of a reference haplotype r, as following:

$$\frac{\Sigma_j(Geno_{ij} - Geno_{rj})}{p},$$

with $Geno_{ij}$ and $Geno_{rj}$ being the estimated effects of MHC genotypes comprised of haplotypes i and j, and r and j, respectively, and p being the number of pairwise combinations.

RESULTS

MHC distribution in the different lines

Frequencies of MHC genotypes and haplotypes in the ninth and tenth generations for the H, C and L lines are given in tables I and II, respectively. Frequencies of genotypes and haplotypes were significantly ($P < 0.01$) different between lines in the ninth and in the tenth generations. In the C line, all possible ten genotypes were present, with a predominance of the 119-124 B-genotype, and the 119 and 124 B-haplotypes were prevalent. The distribution of MHC genotype and of MHC haplotype in the H line was opposite to the ones in the L line. The 121-121 B-genotype predominated in the H line, whereas the 114-114 and 114-124 B-genotypes were present most in the L line. In the H line, the 121 B-haplotype frequency reached 79% at the expense of the 114 B-haplotype, which tended to disappear. On the contrary, the 121 B-haplotype disappeared between the eighth and the ninth generation in the L line (data not shown). In the L line, the 114 B-haplotype was found most compared to the 124, and especially the 119 B-haplotypes.

Heterozygous animals were in majority in the C line, whereas homozygous animals were most frequent in the H line and to a lesser extent in the L line. This tendency was more pronounced in the tenth generation.

Estimation of MHC genotype effects on the Ab response

Estimates of MHC genotype effects on the Ab response to SRBC are given in table III. The overall effect of MHC genotypes was greater in the selected lines than in the C line, and the total genetic variance explained by MHC genotypes was greater in the H and C lines than in the L line. This result was mainly due to the extreme estimate values of

the rare genotypes such as the low estimate value of the 114-124 B-genotype in the H line, which concerned only three animals. Likewise, the 119-119 and 124-124 B-genotypes estimates in the H line were significantly higher than most of the other estimates, but were represented by only nine and eight animals, respectively. The ranking of genotypes according to their estimates of effects on the Ab titer differed between lines; however, the differences were smaller between the C line and the L line than between the C line and the H line. Comparisons of genotype effects between the H and L lines were difficult because not all the genotypes found in the L line were present in the H line, and *vice versa*. No significant changes in the estimate were observed when taking other input values for heritability between 0.2 and 0.4 (data not shown).

Table I. B-genotype frequencies (in %) in the H, C and L lines of the ninth and tenth generations, ordered by decreasing number of animal per genotype in the L line.

	Line								
	H		C		L				
	Generation		Generation		Generation				
Genotype	9	10	9	10	9	10	ALL[1]	ALL	ALL
114-114	0	0	2.9	4.6	33.8	44.6	0	13	256
114-124	0.5	0.5	12.2	4.0	38.1	29.1	3	31	234
114-119	0	0	10.7	11.2	11.5	8.1	0	39	69
124-124	2.2	0	20.5	7.9	8.9	10.5	8	54	64
119-124	2.2	8.7	15.6	26.3	7.7	5.0	26	72	45
119-119	2.5	0	6.4	9.2	0	2.7	9	27	7
114-121	7.4	0	3.4	8.5	0	0	27	20	0
121-124	12.7	11.6	8.8	14.5	0	0	70	40	0
119-121	12.7	12.1	12.2	9.2	0	0	71	39	0
121-121	59.8	67.1	7.3	4.6	0	0	356	22	0
ALL	363	207	205	152	417	258	570	357	675

[1] ALL: number of animals.

Table II. Frequencies (in %) of B-haplotype and of heterozygote animals in the H, C and L lines of the ninth and tenth generations, ordered by decreasing haplotype frequency in the L line.

	Line					
	H		C		L	
	Generation		Generation		Generation	
Haplotype	9	10	9	10	9	10
114	4.0	0.2	16.1	16.4	58.6	63.2
124	9.9	10.4	38.8	30.3	31.8	27.5
119	9.9	10.4	25.6	32.6	9.6	9.3
121	76.2	79.0	19.5	20.7	0	0
Hetero[1]	35.5	32.9	62.9	73.7	57.3	42.3

[1] Hetero: % of heterozygote animals within lines.

Table III. Estimates of B-genotype effect on the Ab response to SRBC in the H, C and L lines of the ninth and tenth generations.

Line					
H		C		L	
Genotype	Estimate	Genotype	Estimate	Genotype	Estimate
114-124	-2.89^{a}	114-114	-0.44^{a}	114-124	-0.80^{a}
119-124	-0.27^{b}	121-121	-0.23^{a}	114-119	-0.36^{ab}
121-124	-0.10^{b}	114-119	-0.17^{a}	114-114	-0.35^{ab}
121-121	0.00^{b}	114-124	0.00^{a}	124-124	0.00^{b}
119-121	0.28^{b}	119-119	0.26^{ab}	119-124	0.44^{b}
114-121	0.57^{bc}	114-121	0.35^{ac}	119-119	1.40^{b}
119-119	2.14^{cd}	124-124	0.39^{ac}		
124-124	3.13^{d}	119-121	0.71^{ac}		
		119-124	1.10^{bc}		
		121-124	1.56^{c}		
$Pr > F$[1]	0.0002		0.0235		0.0074
σ^2_G	0.38		0.31		0.13

^{a,b,c,d}: Estimates with different superscripts indicate differences ($P < 0.05$) between genotypes within lines.
[1] $Pr > F$ indicates the overall effect of B-genotypes within lines.
$\sigma^2_G = \Sigma_i\, p_i\, (est_i - \overline{est})^2$: total genetic variance arising from the MHC loci in generation 9; p_i and est_i are, respectively, the frequency in the ninth generation and the estimate of the i^{th} genotype, and $\overline{est}$ is the average genotype estimate ($\Sigma_i\, p_i\, est_i$).

Estimation of heterozygote superiority

Estimates of heterozygote superiority for each available combination and overall lines are given in table IV. In the C line, a moderately positive general effect of heterozygous genotypes was demonstrated. This positive effect appeared in the 119-124 B-genotype, and were marked in the 121-124 B-genotype. In the L line, a general heterozygous disadvantage was not significant. This negative effect, however, was only significant for the 114-124 B-genotype. In the H line, not all the heterozygous combinations could be evaluated because of missing genotypes. In the H line, there was a significant negative effect of heterozygous genotypes overall, and for the 121-124 and the 119-124 B-genotypes.

Table IV. Heterozygote *versus* homozygote superiority (± standard error) in the H, C and low L lines of the ninth and tenth generations.

	Line		
Genotype	H	C	L
114-119		-0.79 ±0.58	-0.89 ±0.56
114-121		0.68 ±0.70	
114-124		0.03 ±0.57	-0.63** ±0.24
119-121	-0.79 ±0.47	0.70 ±0.52	
119-124	-2.91** ±0.72	0.78* ±0.40	-0.26 ±0.65
121-124	-1.66** ±0.50	1.48** ±0.48	
ALL[1]	-1.79** ±0.42	0.60* ±0.31	-0.59 ±0.38

*, **: significant superiority at the 0.05 and 0.01 levels, respectively. [1] ALL indicates the overall superiority within lines.

Estimation of MHC haplotype effects on the Ab response

In the C and L lines, all possible combinations of haplotypes were present. Therefore, in these lines, the choice of a reference haplotype did not affect either the ranking, or the value of the differences between haplotype estimates. In the H line,

haplotype effects were not estimated because it was not possible to write a linear combination of genotypes, which would estimate the difference between two haplotypes. The estimated Ab titer of the 114 B-haplotype was significantly lower than the estimate of the 119 B-haplotype in the L line (table V). In the C line, the estimated Ab titer of the 114 B-haplotype was significantly lower than the estimates of the 121 and 124 B-haplotypes in the C line.

Table V. Estimates of B-haplotype effect on the antibody response to SRBC in the C and L lines of the ninth and tenth generations.

Line			
C		L	
Haplotype	Estimate	Haplotype	Estimate
114	-0.54^{a}	114	-1.00^{a}
119	0.00^{ab}	124	-0.61^{ab}
121	0.12^{b}	119	0.00^{b}
124	0.28^{b}		

The 119 B-haplotype was taken as a reference. ^{a,b}: Estimates with different superscripts indicate differences ($P < 0.05$) between genotypes within lines.

Relationship between the effects of MHC types on Ab response and their frequency

To determine if the differences in MHC genotype and haplotype distribution between the lines could be explained by differences in genotype or haplotype effect on the selected trait, frequencies of MHC types and their estimated effects on the Ab titer were compared. These relationships should be considered globally, since many estimates of genotype and haplotype effects were not significantly different from each other.

The ranking of estimates of haplotype effects on the Ab response in the L line, was in total agreement with the distribution of these haplotypes in the selected lines. Likewise, the ranking of the 114, 119 and 121 B-haplotype effects estimated in the C line could explain the haplotype distribution in the selected lines. On the contrary, the estimate of the 124 B-haplotype effect was high in the C line; however, this estimate was not

significantly different from the estimates of the 121 and 119 B-haplotype effects.

The relationships between genotype frequency in the H and L lines and estimates of their effects within line and from the C line are shown in figures 1 and 2 respectively. The rare genotypes in the H line were not considered in figure 1a. In the H line, the predominance of the 121-121 B-genotype did not permit assessment of any relationship (fig 1a); besides none of the estimates of the genotype effects represented were significantly different from each other. The relationships in the L line were quite consistent (fig 1b). With the exception of the 121-121 B-genotype, genotypes present in the L line had globally lower estimated effect values in the C line than the genotypes present in the H line (fig 2).

FIGURE 2. Frequencies of B-genotypes in the high (H,●) and low (L,✚) lines of the tenth generation, according to their effect on the Ab titer to SRBC, estimated from the control (C) line.

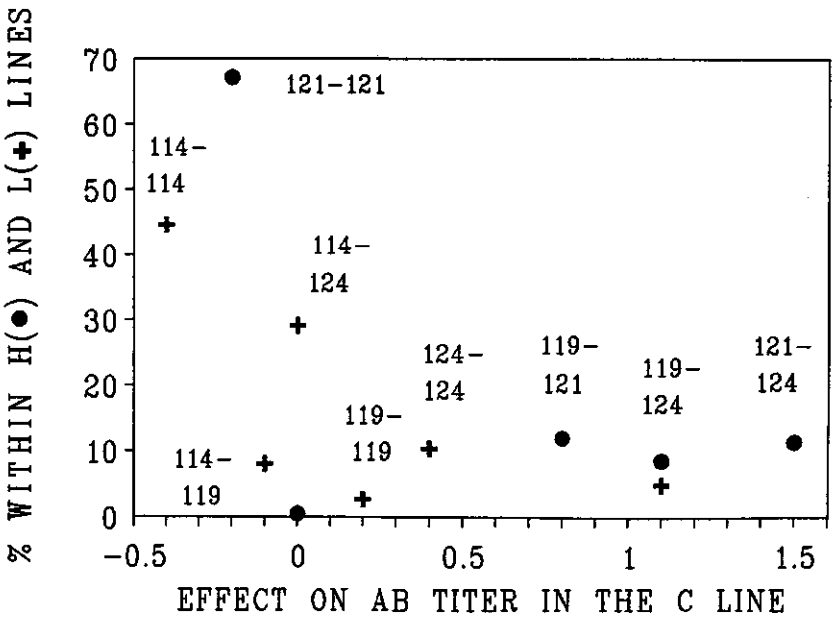
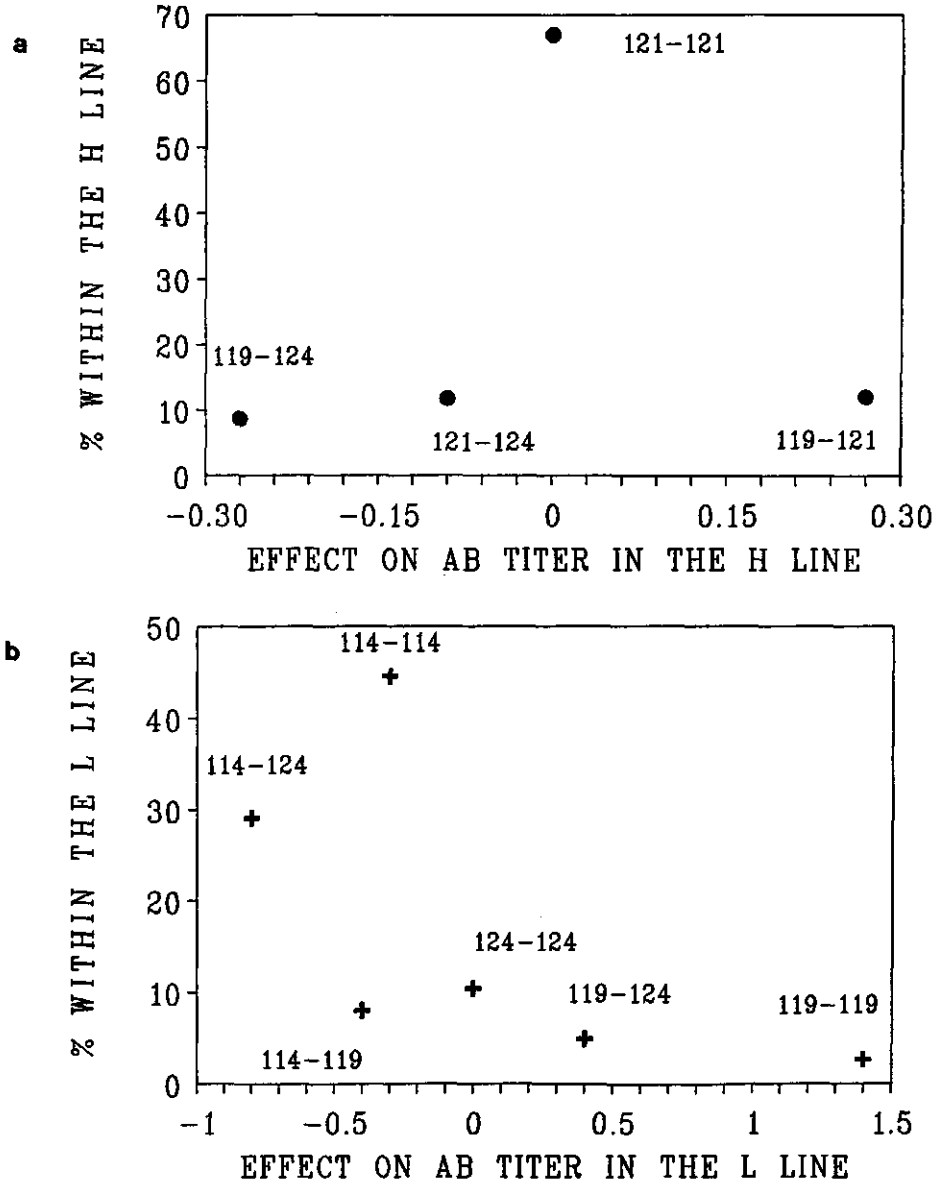


FIGURE 1. Frequencies of B-genotypes in the high (H,●) and low (L,+) lines of the tenth generation, according to their effect on the Ab titer to SRBC, estimated from the high (fig 1a) and the low (fig 1b) lines, respectively.



DISCUSSION

Changes of gene frequency may be due to genetic drift, difference in fitness of certain genotypes, or, in case of selection, due to direct effect or linkage with genes affecting the selected trait (Falconer, 1989). Even after ten generations, genetic drift is not likely to explain such dramatic changes of MHC type frequency in opposite directions. Moreover, previous genetic analysis of nine generations did not show any apparent genetic drift, and inbreeding, which affects genetic drift, was low (Pinard et al, 1992). Associations between MHC types and fitness traits have been demonstrated in avian (Gavora et al, 1986; Nordskog et al, 1987) and mammalian species (Melnick et al, 1981; Ostergard et al, 1989; Gautschi and Gaillard, 1990). Therefore, a possible effect of natural selection cannot be excluded. This, however, cannot explain the opposite MHC type distributions between the H and L lines. The significant differences in effect of the MHC genotype on the selected trait are evidence for a direct or closely linked effect of MHC genes on the Ab response to SRBC.

The MHC type frequencies were not measured in the initial base population or in the first generations of selection. However, the control line was produced from the base population by random mating and displayed in the tenth generation all haplotype combinations, whereas the selected lines presented divergent distributions of MHC types. Given the relatively low level of inbreeding in the C line, it seems thus reasonable that the frequencies in the C line represent the distribution of MHC types in the base population, and that the MHC type frequencies have changed in the selected lines.

Changes in MHC gene frequency, or at least, differences in MHC type distributions between lines selected for immune responsiveness or disease resistance have been reported (Gavora et al, 1986; Heller et al, 1991). In an experiment similar to ours with chickens selected for high and low immune response to SRBC, differences in allelic frequency in six alloantigen systems including the B-system were found in an analysis of data from the generations ten to 13 (Dunnington et al, 1984; Martin et al, 1990). Interestingly, these authors reported that the most frequent B-haplotype in the H line was the 21, which shares B-F antigens with the 121 B-haplotype, which was also predominant in our H line (Pinard and Hepkema, 1992 (submitted)). Typing for MHC antigens in lines

of mice divergently selected for Ab response to SRBC (Biozzi et al, 1979) also revealed two distinct haplotypes in the two lines (Colombani et al, 1979).

Estimation of MHC genotype effect and of heterozygote advantage produced different results between the lines. Immune responsiveness to various antigens like SRBC has been demonstrated to be influenced by non-MHC as well as by MHC genes (Palladino et al, 1977; Gyles et al, 1986; Kim et al, 1987; Lamont and Dietert, 1990). From their selected lines of chickens, Dunnington et al (1989) obtained backcrosses, which contained three different genotypes against either a H or a L background. They showed significant interactions between MHC and the background genome. Moreover, the heterozygous genotype was superior in the H background only. Interactions between MHC genes and background genes have been extensively demonstrated, for example in resistance to Marek's disease (Schierman and Collins, 1987; Steadham et al, 1987). In these experiments, heterozygote advantage resulted from genetic complementation between both MHC and non-MHC genes.

In segregating populations, the estimation of single gene effects can lead to biased results because of the likely confounding effects between the marker gene and the polygenes (Bentsen and Klemetsdal, 1991). Selection is an extra source of bias because the animals being selected are likely sharing advantageous alleles for both the marker gene and the polygenes. Kennedy et al (1992) showed that unbiased estimates of a single gene effect can be obtained by mixed model analysis from a selected population if all the genotypes are known. Biased estimates were unfortunately found in our experiments, because the genotypes were not determined in the early generations. We chose to use the data complete for both the Ab titer and the genotypes from the last two generations, instead of using data from all generations with an unknown genotype. Indeed, Carnier and Arendonk (1992) demonstrated by simulation that including observations in previous generations of which genotype information was missing resulted in larger biases. In our estimation, bias due to selection could not be eliminated by the use of the complete relationship matrix only. This bias might have contributed to the differences in genotypic effects observed between the lines.

The present and previous results (Pinard et al, 1992) are in agreement with a polygenic control of the antibody response to SRBC. Moreover, one of the loci involved might be part of, or linked to, the B-complex. However, the linkage and the nature of the

interactions between MHC or MHC-linked genes and other immune response genes are not known. Besides, during ten generations of multiple matings, recombinations between MHC-linked and other immune response genes might have occurred, causing altered linkage (Pevzner et al, 1978; Lamont, 1989).

In conclusion, results of the estimation of MHC effect from selected population should be considered with caution, especially when the genotypes are not known in all the generations. In our experiment, estimation of MHC effect may be obtained from the control line, providing a larger number of animals; indeed, the number of animals per genotype, especially homozygous ones, was here too limited to insure a reliable evaluation. Alternatively, one could study a F2 population that will be produced from the high and low lines.

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CHAPTER 4

EFFECT OF MAJOR HISTOCOMPATIBILITY COMPLEX TYPES IN F1 AND F2 CROSSES OF CHICKEN LINES SELECTED FOR IMMUNE RESPONSIVENESS.

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EFFECT OF MAJOR HISTOCOMPATIBILITY COMPLEX TYPES IN F1 AND F2 CROSSES OF CHICKEN LINES SELECTED FOR IMMUNE RESPONSIVENESS.

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ABSTRACT

Lines of chickens selected for nine generations for high (H) and low (L) antibody (Ab) response to sheep red blood cells (SRBC) were crossed to produce F1 (n=761) and F2 (n=1033) populations. All animals were typed for major histocompatibility complex (MHC) B-types. Effects of MHC genotypes and haplotypes on the Ab titer to SRBC were estimated. The MHC genotypes and remaining genotype explained about 3.5% and 31% of the total variation of the Ab titer in the F2, respectively. Estimates of MHC effects in the F2 were similar to estimates in the selected lines. The 119 and 121 B-haplotypes were associated with a significantly higher response than the 114 and 124 B-haplotypes. These results confirm the hypothesis that changes in B-type distribution observed in the selected lines could be related to a direct or closely linked effect of MHC on the immune response.

Key words: chicken, humoral response, selection, F2, major histocompatibility complex

INTRODUCTION

There is accumulating evidence that disease resistance and immune response are under genetic control in most species, providing the bases for an improvement by direct selection for the trait of interest; moreover, the use of markers might add to the efficiency of selection (Shook, 1989; Weller and Fernando, 1991). But in the latter option, relationships between marker genes and the trait of interest have to be clearly established. Studies on relationships between major histocompatibility complex (MHC) types and immune traits or disease resistance have shown variability in strength and nature of association (Schierman and Collins, 1987; Van der Zijpp and Egberts, 1989). Inconsistencies might be due to several reasons: (a) the MHC is not affecting directly the trait and some crossing over has occurred between the MHC and immune response genes, leading to opposite effects, (b) the MHC is directly involved but there are epistatic effects with other background genes and/or significant genotype environment interactions, (c) only a few MHC types are present per study, so that the same haplotypes differ in relative performance (good or poor) in different populations, (d) different and even inappropriate statistical methods might have been used, especially when animals are related.

High (H) and low (L) lines of chickens have been produced by divergent selective breeding for primary antibody response to sheep red blood cells (SRBC) (Van der Zijpp et al, 1988; Pinard et al, 1992a). After ten generations, the H and L lines revealed a diverging distribution in MHC types, compared to the random control line; moreover, MHC types were responsible for a significant part of variation of the immune response (Pinard et al, 1992b). However, MHC genotypes were not known in early generations so that estimates of the MHC effect might be biased, even when using all family information (Kennedy et al, 1992). Moreover, the number of animals for some genotypes was limited. Therefore, a study involving crosses between the H and L lines was required to confirm the MHC association.

The objectives of this experiment were to produce F1 and F2 crosses from lines of chickens selected for high and low antibody response to SRBC, and to estimate the MHC genotype and haplotype effects on the immune response against a random background.

MATERIALS AND METHODS

Crossing of selected lines

Chickens were selected, from an ISA Warren cross base population, for high (H) or low (L) total antibody (Ab) titer, five days postprimary immunization with 1 mL 25% sheep red blood cells (SRBC) (Van der Zijpp et al, 1988; Pinard et al, 1992a). From the ninth generation, 26 males and 55 females of the H line were mated with 53 females and 31 males of the L line, respectively, to produce 761 F1 animals. From the F1 population, 243 females and 202 males were used to produce 1033 F2 chicks. Parents of the F1 and F2 populations were chosen from as many different families as possible, and were mated at random, providing in F2 about 100 chicks for each of the ten MHC genotypes (see below). Immunization with SRBC was performed on F1 and F2 animals identically as in the selected lines, and Ab titers against SRBC five days postprimary immunization were recorded. The vaccination schedule applied to F1 and F2 chicks was identical to the one performed during the selection. However, the housing system and environment differed: whereas selected animals were housed in cage pens, F1 and F2 birds were kept on the floor on two different farms, respectively.

Typing for MHC haplotype

Major Histocompatibility Complex haplotypes were determined by direct haemagglutination, using alloantisera obtained from the lines. Four serotypes, provisionally called B^{14}, B^{19}, B^{21}, and B^{24} were identified previously in the selected lines (Pinard et al, 1991; Pinard and Hepkema, 1992). A MHC genotype was defined as the combination of two haplotypes. Serological typing was performed on all the F1 and F2 chicks and segregation of the haplotypes was checked for consistency within families; inconsistent data (3% of the data) were removed from the analysis.

Statistical analysis

Effects of MHC genotype on the Ab response were estimated in the F1 and F2 populations by PEST (Groeneveld, 1990; Groeneveld and Kovac, 1990) using the following mixed model:

$$Ab_{ijk} = \mu + sex_i + MHC_j + U_{ijk} + e_{ijk}$$

Where:

Ab_{ijk} = the Ab titer of the k^{th} chick,
μ = a constant,
sex_i = the fixed effect of the i^{th} sex of the chick,
MHC_j = the fixed effect of the j^{th} MHC genotype,
U_{ijk} = the random additive genetic effect on the Ab titer in the k^{th} chick and
e_{ijk} = a random error.

The sex effect corrected for a higher Ab response to SRBC in females than in males. All relationships from the base population until the F1 and F2 crosses were used in the analysis of the F1 and F2 data, respectively. The mixed model was applied assuming a heritability of 0.31, as estimated previously from data of all lines (Pinard et al, 1992a).

Differences between genotypes within lines were tested by an F value calculated by PEST, which allows to use all relations between animals. The overall effect of genotypes was estimated by testing, jointly, n-1 independent differences between genotypes, with n being the number of genotypes.

Heterozygote superiority was estimated for each available combination by testing the difference between the heterozygote genotypes and the average of their homozygous counterparts. The overall heterozygote superiority was estimated by testing the difference between all the heterozygote genotypes and the average of their homozygous counterparts.

The haplotype effect was estimated by three methods. In method I, the effect of haplotype i was estimated by testing the difference between genotype combinations, comprised of the haplotype i and their counterparts, comprised of a reference haplotype r, as follows:

$$\frac{\Sigma_j(\text{Geno}_{ij} - \text{Geno}_{rj})}{p},$$

with Geno_{ij} and Geno_{rj} being the estimated
effects of MHC genotypes comprised of haplotypes i and j, and r and j, respectively, and p being the number of pairwise combinations. Methods II and III were applied in the following haplotype models, as adapted from Østergard (1989):

$$\text{Ab}_{ijl} = \mu + \text{sex}_i + \Sigma_j\beta_j\text{Haplo}_j + U_{ijl} + e_{ijl} \quad \text{(method II)}$$
$$\text{Ab}_{ijkl} = \mu + \text{sex}_i + \Sigma_j\beta_j\text{Haplo}_j + \Sigma_k\Gamma_k\text{Comb}_k + U_{ijkl} + e_{ijkl} \quad \text{(method III)}$$

Where β_j is the linear regression coefficient on Haplo_j, which is the number of the j^{th} MHC haplotype (2=homozygous, 1=heterozygous or 0=absent) in the l^{th} chick, Γ_k is the linear regression coefficient on Comb_k, which is the k^{th} heterozygous combination, and all the other terms are as previously described.
In the F1 cross, only method I was applied, whereas all three methods were compared in the F2 population, which provided all possible haplotype combinations in equal numbers of animals.

RESULTS

Antibody titer distribution in the F1 and F2 populations

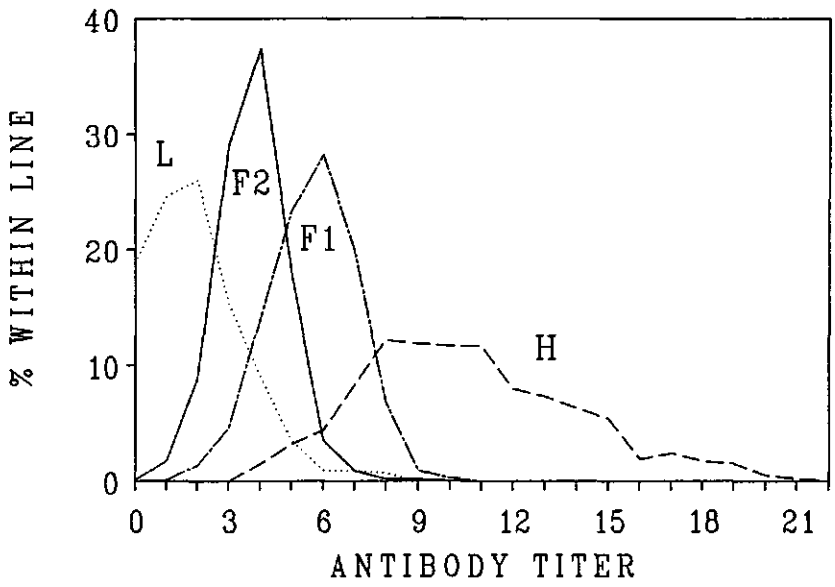
Antibody titer distributions in the H and L lines of the ninth generation, and in the F1 and F2 crosses are shown in figure 1, and mean titers are given in table 1. The F1 cross did not show any positive heterosis effect, and the titer of the cross between L line females and H line males was even lower (5.85) than the mean parent value (9.06). The Ab titers were more normally distributed in the F1 and F2 crosses than in the selected lines, but the F2 population did not show a greater variation of titers than the F1 cross.

TABLE I. Means ± Standard deviations of Ab titers in the high and low lines of the ninth generation, and their F1 and F2 crosses.

	Ab titer		
Line	Whole line	Male parent[1]	Female parent[1]
L	1.94 ± 1.57	0.00 ± 0.00	2.89 ± 1.71
H	10.62 ± 3.38	15.23 ± 1.55	11.67 ± 3.46
$H_{♂} \times L_{♀}$[2]	5.85 ± 1.34		
$L_{♂} \times H_{♀}$[2]	5.46 ± 1.44		
F1[3]	5.66 ± 1.64	5.47 ± 1.42	5.83 ± 1.34
F2	3.77 ± 1.13		

[1]Males and females from the line, used to produce the next cross.
[2]F1 reciprocal crosses
[3]Whole F1

FIGURE 1. Distribution of Ab titers to SRBC of the high (H,--) and low (L,··) lines of the ninth generation, H x L = F1 (-·) and F1 x F1 = F2 (——) crosses.



MHC distribution in the F1 and F2 populations

Numbers of animals per MHC genotype in the F1 and F2 crosses are given in table II. Sexes were equally represented in each class. It was not possible to obtain homozygous 121-121 animals in the F1 cross because the 121 B-haplotype was not present in the L line of the ninth generation (Pinard et al, 1992b).

TABLE II. Number of animals per B-genotype in the F1 and F2 crosses.

	Cross	
Genotype	F1	F2
114-114	65	96
114-119	87	108
114-121	201	100
114-124	50	101
119-119	50	97
119-121	88	105
119-124	75	110
121-121	0	103
121-124	88	108
124-124	57	105
ALL	761	1033

Estimation of MHC genotype effects on the Antibody response

Estimates of effects of MHC genotype on the Ab response to SRBC in F1 and F2 animals are given in table III. The overall effect of MHC genotypes was greater in the F2 than in the F1 population. The range of estimates was higher in the F1 than in the F2 population, but the standard errors of differences between genotypes were half as large in the F2 than they were in the F1 cross. The ranking of genotypes according to their Ab titer estimates did not differ greatly between the two populations; only the 124-124 and the 114-121 B-haplotypes showed relatively low estimates, and the 119-119 B-genotype a relatively high estimate in the F1 compared to those in the F2 animals. No significant changes in the estimate were observed when taking other input heritability values between

0.2 and 0.4 (data not shown). In the F2, the distributions of Ab titers within genotypes were normal and ranged between those of the 114-124 and 119-121, as shown in figure 2.

Comparisons of genotype effects estimated in F2 with their estimation in the H, C and L lines (Pinard et al, 1992b) are shown in figure 3. Results of the F2 were more in agreement with those of the selected lines than of the C line.

An attempt to estimate the relative importance of the MHC genotype and the remaining genotype on the variation of the Ab titer in the F2 was performed by comparing the coefficient of determinations using different models (table IV). When used solely in the model, only 4.4% of the total variation were explained by the MHC genotype effect, which could still partly confounded with the effects of the sex and of U_k. When using the full animal model, the MHC genotype contributed an additional 2.5% of variation. The R^2 value of 31.1 when putting only U_k as an effect was close to the input heritability (0.31) as expected.

TABLE III. Estimates of B-genotype effect on the Ab response to SRBC in the F1 and F2 crosses.

Cross			
F1		F2	
Genotype	Estimate	Genotype	Estimate
124-124	-0.94^{a}	114-124	-0.50^{a}
114-124	-0.82^{a}	114-114	-0.28^{ab}
114-114	-0.79^{a}	121-124	-0.18^{abc}
114-121	-0.48^{ab}	124-124	-0.17^{abcd}
121-124	-0.47^{abc}	119-119	-0.08^{bcd}
114-119	-0.03^{bcd}	114-119	-0.05^{bcd}
119-124	0.00^{bcd}	119-124	0.00^{bcde}
119-121	0.07^{cd}	114-121	0.11^{cde}
119-119	0.45^{d}	121-121	0.21^{de}
		119-121	0.35^{e}
$Pr > F^{1}$	0.0005		< 0.0001

^{a,b,c,d}: Estimates with different superscripts indicate differences ($P < 0.01$) between genotypes within cross.
$^{1}Pr > F$ indicates the overall effect of B-genotypes within cross. Standard errors of differences between genotypes were between 0.28 and 0.32 in F1, and 0.16 in F2.

TABLE IV. Contributions of the effects of the MHC genotypes and the animal value to the total variance in Ab titer in the F2.

Model[1]	$R^{2\,2}$
$Ab_{jk} = \alpha + MHC_j + e_{jk}$	4.4
$Ab_k = \alpha + U_k + e_k$	31.1
$Ab_{ik} = \alpha + sex_i + U_{ik} + e_{ik}$	31.7
$Ab_{ijk} = \alpha + sex_i + MHC_j + U_{ijk} + e_{ijk}$	34.2

[1] The factors in the model are as previously described

[2] $R^2 = 1 - \frac{N - p}{N - 1} \times \frac{\sigma^2_{e\ residual}}{\sigma^2_{phenotypic}}$

where N is the number of observations and p is the degree of freedom of the model.

FIGURE 2. Distribution of Ab titers to SRBC of the 114-124 (··), 119-119 (——) and 119-121 (--) genotypes in the F2.

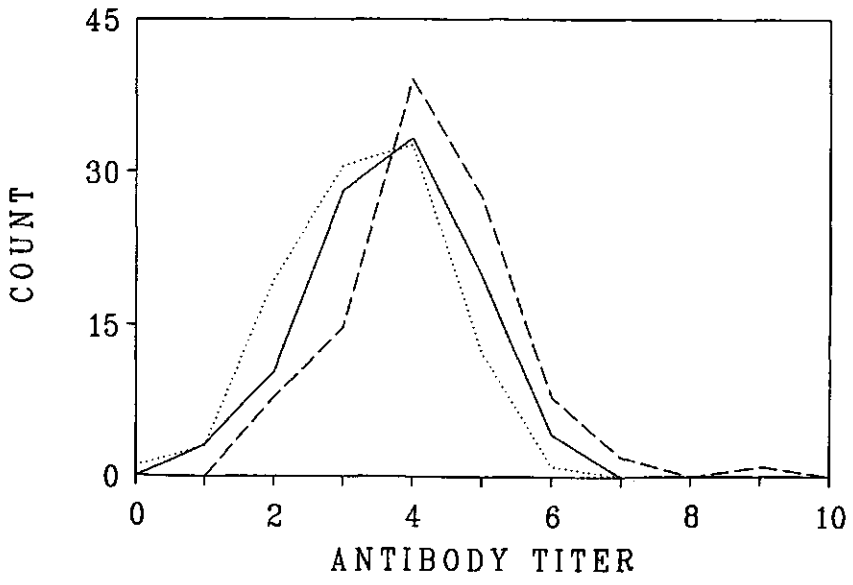
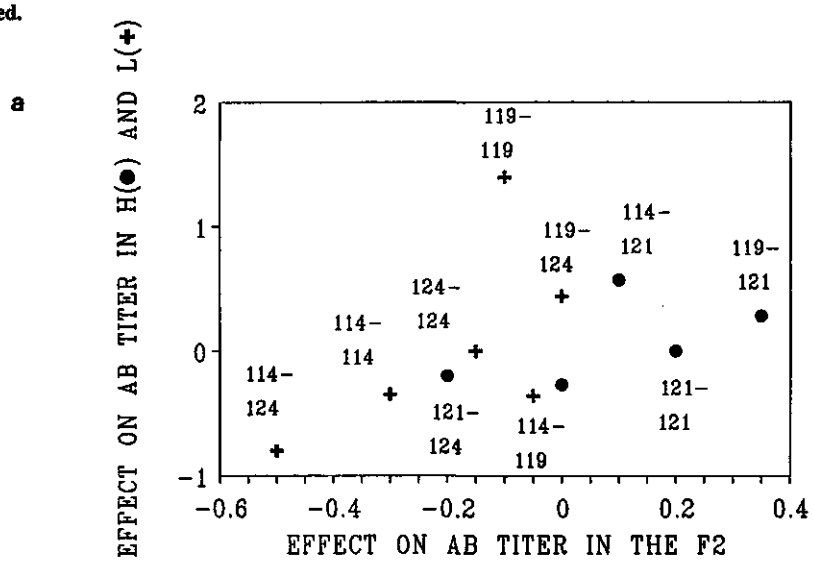
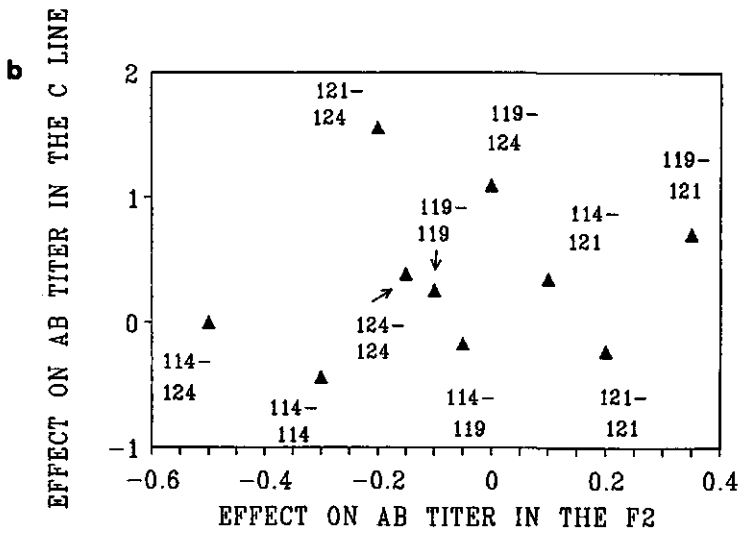


FIGURE 3. Effects of MHC genotypes on Ab titers to SRBC estimated in the high (H,●) and low (L,+) lines (Fig 3a) and in the control (C,▲) line (Fig 3b) according to their effects on Ab titers to SRBC estimated in the F2. Results of the H, C and L lines are from Pinard et al (1992), and rare genotypes in the H line were not considered.





Estimation of heterozygote superiority

In the F1 population, no significant effect of heterozygote superiority, overall or for any available combination, was found (data not shown). No significant effect of overall heterozygote superiority was shown in F2 animals either (table V); however, the 114-124 and 119-121 B-genotypes demonstrated a significant heterozygous disadvantage and advantage, respectively.

TABLE V. Heterozygote *versus* homozygote superiority (± standard error) in the F2 cross.

Genotype	Hetero sup.
114-119	0.13 ±0.13
114-121	0.15 ±0.13
114-124	-0.28* ±0.14
119-121	0.29* ±0.13
119-124	0.12 ±0.13
121-124	-0.20 ±0.13
ALL[1]	0.04 ±0.07

[1] ALL indicates the overall superiority. *: significant superiority at the 0.05 level.

Estimation of MHC haplotype effects on the Ab response

Results of the estimation of MHC haplotype effect on the Ab titer in the F1 and F2 populations, using method I are given in table VI. In the F1 population, the 119 B-haplotype was significantly associated with the highest estimate, whereas in the F2 animals, the estimated Ab titers of the 119 and 121 B-haplotypes were significantly higher than for the 114 and 124 B-haplotypes. Using method II in the F2 population did not significantly change the relative values of haplotypes. Haplotype effects estimated by method III were in fact their additive effects in homozygous combinations, whereas the specific combination effects were simply equal to heterozygous effects as given in table V (data not shown).

TABLE VI. Estimates (± standard errors) of B-haplotype effect on the antibody response to SRBC in the F1 and F2 crosses.

cross							
F1		F2					
Method I		Method I		Method II		Method III	
Haplo	Est	Hapl	Est	Haplo	Est	Haplo	Est
124	0.00^{a}	124	0.00^{a}	124	0.00^{a}	114	-0.14^{a}
114	0.03^{a} ±0.13	114	0.03^{a}	114	0.01^{a}	124	-0.09^{ab}
121	0.22^{a} ±0.19	119	0.27^{b}	119	0.18^{b}	119	-0.04^{ab}
119	0.68^{b} ±0.15	121	0.33^{b}	121	0.28^{b}	121	0.11^{b}

The 124 B-haplotype was used as a reference. ^{a,b}: Estimates with different superscripts indicate differences ($P < 0.01$) between haplotypes within crosses. Standard errors of differences between haplotypes in the F2 were all equal to 0.08, 0.07 and 0.08 by using methods I, II and III, respectively.

DISCUSSION

When parental lines are crossed, the amount of heterosis shown by the F1 may be defined as its deviation from the mid-parent value (Falconer, 1989). Crossing effects are due to differences in the allelic frequencies between the two parental lines. In this experiment, the two lines that were crossed came from the same base population. However, after nine generations of selection, they differed greatly for MHC haplotype frequency and likely for other immune response genes associated with the response to SRBC (Pinard et al, 1992b). No heterosis was demonstrated here. Nevertheless, the reciprocal crosses showed similar Ab titer values although their respective mid-parent values differed, indicating maternal or sex-linked effects. In crosses of lines of mice at their selection limit for Ab response to SRBC, positive heterosis was shown and was interpreted as partial dominance of the character 'high responder' (Biozzi et al, 1979). In a similar experiment, crossing of White Leghorn chicken lines which were selected for high and low immune response to SRBC for three generations showed a positive heterosis effect when

compared with the parental lines (Siegel and Gross, 1980). After nine generations, the same line crosses were lower than their mid-parent average but intermediate between the whole parental lines (Ubosi et al, 1985). In our lines, environmental effects were responsible for more than two titer points of variation in Ab titer during the selection (Pinard et al, 1992a). Therefore, if environmental effect and variance cannot be accurately quantified, populations kept separately should not be compared.

Because of limitations in the data obtained through selection and the expected bias in estimates of genotype effects from selected lines (Kennedy et al, 1992; Pinard et al, 1992b), a F2 was produced. In fact, results of estimation of genotype effects in the F2 were more similar to the estimated effects in the selected lines than in the C line (figure 3), giving credibility to the analysis performed in the selected lines. The average genetic value of the C line did not change during the selection (Pinard et al, 1992a) and the C line displayed, as the F2, a random background. However, the F2 background has a relatively great frequency of "high" and "low" immune response genes, whereas the C background had "low", "average" and "high" genes from the base population. Thus, besides the fact that estimation of genotype effects in the C line could be hampered by low numbers of animals, differences of effects between the F2 and the C line may be interpreted as interaction between MHC and other immune response genes. Moreover, linkage disequilibrium created in the selected lines between MHC genes and immune response genes may not have disappeared completely in the F2.

How do the results of the F2 contribute to the understanding of the role played by MHC haplotypes during selection ? In the Biozzi lines of mice at their selection limit, analysis of F2 cross showed that about ten loci were involved, and among them, the MHC locus was responsible for 12% of the interline difference. Moreover, the MHC haplotypes found in the H and the L lines segregated, respectively, with a higher and a lower immune response (Mouton et al, 1979). In our experiment, the selection limit was not reached and selected lines were not homozygous for either the 'high' or 'low' responder character. Nevertheless, the MHC haplotypes most frequent in the L line (114 and 124) and in the H line (119 and 121) were associated in the F2 with the lowest and highest Ab titer, respectively. In addition to the previous estimates (Pinard et al, 1992b), we can definitively say that the changes of MHC type frequency observed in the selected lines were not the result of chance, but could be explained by a direct or closely linked effect of MHC types

on the selected immune response. However, it is clear that the magnitude of MHC effects, as measured here could not fully explain the interline difference. We estimated that approximatively 3.5% of the total variation only could be attributed to the MHC whereas the part of the total genetic variation was 31% (heritability value).

Associations of MHC with immune response to SRBC have been shown in chickens (Scott et al, 1988; Loudovaris et al, 1990), mice (Mouton et al, 1979) and miniature pigs (Mallard et al, 1989). These studies, including ours, cannot discriminate between direct and linked effects of MHC; however, immunological knowledge of MHC can support the hypothesis of a direct involvement. When injected, the T-dependent SRBC antigens are phagocytized and processed by macrophages, and finally presented to T-helper cells, inducing, in collaboration with B-cells, the production of Ab against SRBC (Biozzi et al, 1984). The T-B cell interaction has been shown to be in chickens, as in mammalian species, MHC class II (B-L) restricted as is the presentation of processed peptides to T-cells (Vainio et al, 1987). In Biozzi mice, where MHC played a major role in regulation of the immune response, differences in the level of Ab titer were shown to be based upon the modification of antigen processing and presentation by macrophages (Biozzi et al, 1984). Efficiency of the response may be related to the varied ability of MHC molecules to bind and present antigens to T-cell receptors (Watts and Mc Connell, 1987; Buus et al, 1987). Thus, to trigger the immune response, MHC protein binding ability, while necessary, is not solely sufficient and combines with the T-cell repertoire (Grey et al, 1989). Finally, Kaufman and Salomonsen (1992) proposed some models for a possible role of class IV (B-G) genes in the selection of B-cells. Positive and negative complementation in these different paths could explain, respectively, the heterozygous advantage and disadvantage observed for the combinations of the two "best" (119 and 121) and the two "worst" (114 and 124) B-haplotypes, regarding their effect on antibody response to SRBC.

In the case of non-additivity of some MHC-linked genes, a genotype model should be preferred because it is the most complete and allows parallel estimation of the general and specific heterozygous effects. In the F2, all possible haplotype combinations were present in a balanced design. This is often not the case; a genotype model should be, then, also used to avoid the risk of having haplotypes effects completely dominated by one genotype. However, it can be of practical interest to search for favourable alleles, for example in

cattle breeding where only sires are MHC typed and extensively used, by using haplotype models such as type II or adapted from this method (Batra et al, 1989; Lundén et al, 1990). Bentsen and Klemetsdal (1991) proposed a haplotype model including a general heterozygous effect but it is obvious that this hypothesis should be tested before being applied. In the case of additivity, all three haplotype models would give the same estimate; otherwise, the differences between models I and II will depend on the relative value of heterozygous genotypes.

In conclusion, selecting for higher immune response may be achieved by choosing the best specific haplotype combination in a particular genetic stock or line crosses. In many species, it is not easy to utilize the non-additive genetic variation in practice; the typical multiple-line cross which is used in commercial poultry breeding may, however, provide the "necessary" tool.

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CHAPTER 5

EFFECT OF DIVERGENT SELECTION FOR IMMUNE RESPONSIVENESS AND OF MAJOR HISTOCOMPATIBILITY COMPLEX ON RESISTANCE TO MAREK'S DISEASE IN CHICKENS.

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EFFECT OF DIVERGENT SELECTION FOR IMMUNE RESPONSIVENESS AND OF MAJOR HISTOCOMAPTIBILITY COMPLEX ON RESISTANCE TO MAREK'S DISEASE IN CHICKENS.

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ABSTRACT

Lines of chickens selected for nine generations for high (H), low (L) antibody response to SRBC, a randombred control (C) line and a F1 cross between H and L lines were challenged for resistance to Marek's disease (MD). Only hens were challenged at day-old by contact with virulent MD strain K. All animals were serologically typed for MHC erythrocyte antigens. Death from MD occurred between five and 17 weeks of age. Chicks from the L and H line died respectively earlier and later than the C line chicks, whereas F1 animals died at a similar time as the L line chicks. Mortality in the L line (70.1%) was significantly higher than in the C line (42.8%), but mortality in the H line (40.9%) was not significantly lower than in the C line or than in the F1 (47.5%). Effects of MHC genotypes and haplotypes on mortality from MD were estimated within lines with a logistic regression model. Overall effect of MHC was moderate in the H line ($P<.10$) and most significant in the C line ($P<.005$). Effects of MHC genotypes were similar in the H and C line but differed in the L and F1. Heritability of mortality from MD estimated with a threshold 'animal model-like' was overall lines .40, whereas heritability estimated in the H, C and L lines were .45, .51 and .78, respectively. Correlations between estimated breeding values for antibody response to SRBC and mortality from MD varied between lines, sexes, and whether MHC effect was taken into account or not.

Key Words: chickens, Marek's disease, major histocompatibility complex, immune response, selection

INTRODUCTION

Marek's disease (MD) is a neoplastic disease caused by a herpes virus in chickens, and is characterized by a development of lymphoid tumors (Calnek, 1986). Although the incidence of MD has been controlled by vaccination programs, failures to vaccination protection are still reported, which might be reduced by improving genetic resistance to MD (Witter, 1988). Improved genetic resistance to MD has been shown to enhance the effectiveness of vaccination (Gavora and Spencer, 1979) and to improve the resistance to several MD virus strains (Gavora et al., 1990).

Association between resistance to MD and particular alleles of the B blood group (identified later as part of the major histocompatibility complex (MHC)) were first reported by Hansen et al. (1967). The chicken MHC encodes polymorphic cell surface antigens, which play an essential role in regulation of immune response via antigen presentation and restriction of cell cooperation (Bacon and Dietert, 1991). Hansen et al. (1967) showed that animals bearing the B^{19} haplotype died approximately twice more frequently than chicks possessing the B^{21} haplotype. In parallel, Briles et al. (1977) showed that selection for resistance to MD had lead to an increase of the B^{21} haplotype, whereas the B^{19} haplotype was most frequent in the line selected for susceptibility to MD. Various investigations have shown that other B-haplotypes were associated with either resistance or susceptibility to MD and that the effect of MHC depended on the genetic background (Reviewed by Bacon, 1987; Schierman and Collins, 1987).

Resistance to disease may be improved by indirect selection for immune parameters (Van der Zijpp, 1983). Lines of chickens, selected for six generations for high or low antibody response to SRBC, have shown, after a preliminary challenge to MD infection, a higher resistance to MD in the high line than in the low line (Van der Zijpp et al., 1988). However, no control line was challenged and the knowledge on MHC was not present in the lines, so that respective effects of improved immune responsiveness and MHC on resistance to MD could not be clarified.

The objectives of this experiment were (i) to assess the effect of nine generations selection for high and low antibody response to SRBC on resistance to MD in selected and control lines and in a F1 cross, (ii) to estimate the heritability of mortality from MD and

(iii) to estimate the effects of MHC on mortality from MD in the different lines.

MATERIALS AND METHODS

Challenged Lines

Chickens, originating from a ISA Warren cross, were selected for high (H) and low (L) primary antibody (Ab) response to sheep red blood cells (SRBC), and a randombred control (C) line was maintained (Van der Zijpp et al., 1988; Pinard et al., 1992a). From the ninth generation, 16 cocks and 34 hens from the H line, 29 cocks and 56 hens from the C line, and 17 cocks and 34 hens from the L line were used to produce animals of each MHC genotype in sufficient numbers for the Marek's challenge. Parents were all chosen from as many different families as possible. Cocks were not tested. All hens were tested in one house and were obtained from two hatches, at two week interval, from identical parental combinations. Mortality prior five wk was assumed to be due to other causes than Marek's disease and was equal in all lines, leaving on test, respectively, 208, 458 and 274 hens in the H, C and L line.

Challenged F1

From the ninth generation, 19 cocks from the H line and 35 hens from the L line, and 27 cocks from the L line and 52 hens of the H line were used and produced respectively 264 and 367 hens alive after five wk. Parents were all chosen from as many different families as possible. The 631 hens were obtained from two hatches from identical parental combinations.

Marek's Virus Infection and Assessment of Marek's Disease

Parents of the challenged chicks were vaccinated against Marek's disease (MD) at day-old by i.m. inoculation with a virulent live vaccine Delvack, strain CVI 988 (Rispens

et al., 1972). But chicks, which were used in the MD challenge, received no vaccination. Chicks were challenged for MD at day-old by contact with spreader chicks, which had been inoculated at day-old with the virulent MD virus strain K provided by De Zeeuw Laboratories (De Bilt, The Netherlands). Spreader chicks had occupied the floor housing system for 10 days before introduction of chicks to be challenged in a 1:20 ratio. Mortality to MD was identified by gross examination and was recorded daily from five to 17 wk of age. After 17 wk of age, all animals were killed and were grossly examined for MD.

Serological MHC Typing

Major histocompatibility complex haplotypes were determined by direct haemagglutination, using alloantisera obtained from the lines. Four serotypes, provisionally called B^{14}, B^{19}, B^{21} and B^{24} were found previously in the selected lines (Pinard et al., 1991; Pinard and Hepkema, 1992). A MHC genotype was defined as the combination of two haplotypes.

Statistical Analysis

Comparison of the H, C, L lines and F1 for MD Mortality

Mortality by MD in the H, C and L lines and of the F1 were compared by a chi-square test and by calculating their crude Odd's Ratio (OR) (Martin et al., 1987), applying a chi-square test for homogeneity, using the following 2x2 table:

	Dead	Not dead
Line	a	b
Reference	c	d

The characters a,b,c and d represent numbers of cases. The C line was taken as a reference for the calculation of crude OR, but significance of difference between pairs of

lines was tested with the appropriate reference. The crude OR is calculated as (a/c)/(b/d). The crude OR expresses the strength of association between the genetic line and the occurrence of the disease, irrespective of interactions of other variables or confounding. High values (greater than 1) indicate higher risks of MD for the given line as compared to the C line.

Time of Death from Marek's Disease

Week of death from MD was analyzed by the Survival analysis procedure of SAS, LIFETEST (SAS, 1989). The H, C and L lines and the F1 were compared for time of death, and average weeks of death were calculated.

Effects of MHC types on Mortality from Marek's Disease

Genotype effects on MD mortality were estimated separately in the H, C, L lines and in the F1 by multivariate logistic regression (Hosmer and Lemeshow, 1989; Østergård et al., 1989), using the procedure NESTED (Jansen, 1990, 1992) according to the following model:

$$\begin{aligned} \log \text{Odd's}_{ilr} &= \log [p_{ilr}/1\text{-}p_{ilr}] \\ &= \tau + s_i + \text{MHC}_l + e_{ilr} \end{aligned} \qquad \text{(model 1)},$$

with p_{ilr} the probability for the chick r of dying from MD, τ a constant, MHC_l the fixed effect of the MHC genotype l in the chick r, s_i the random effect of the father i of the chick r, and e_{ilr} a random error.

Haplotype effects on MD mortality were estimated separately in the H, C, L lines and in the F1 by multivariate logistic regression according to the following model:

$$\begin{aligned} \log \text{Odd's}_{ilr} &= \log [p_{ilr}/1\text{-}p_{ilr}] \\ &= \tau + s_i + \Sigma_l \text{Haplo}_l + e_{ilr} \end{aligned} \qquad \text{(model 2)},$$

with Haplo_l the fixed effect of the MHC haplotype l (present or absent) in the chick r, and all the other terms are as in model 1. The program NESTED was executed on a logit scale with ten quadrature points. Significance of an effect was tested by a likelihood ratio chi-

square test, comparing the full model and the model without the given effect (Hosmer and Lemeshow, 1989). Hatch effect was not significant and was therefore not included in the model. Although fathers were mated each to two mothers approximately, the mother effect was not included in the model to avoid numerous dummy variables compared to the number of observations and unstable estimates (Hosmer and Lemeshow, 1989). Test of significance between a covariate and the reference was performed by the Wald chi-square test. Obtained estimates (ß) could be changed in OR by exponential transformation (e^{β}). The higher the ß or OR are, the higher the risk of MD is. The 114-114 genotype and 114 haplotype were chosen as references; this choice does not affect the relative value of estimates; but significance of pairwise comparisons were calculated with appropriate reference.

Heritability of Mortality from Marek's Disease

Heritability of mortality from MD was estimated separately in the H, C and L lines and overall lines by restricted maximum likelihood in a threshold model. The threshold model uses a likelihood approach for fitting binomial data. The likelihood (L) for all data can be given as:

$$\text{Log}(L) = \Sigma_r \, y_r \, \Phi(\mu_r) + (1-y_r)[1-\Phi(\mu_r)]$$

where $\Phi(\mu_r)$ is the probability for the chick r of dying from MD, and $y_r = 1$ if the chick r died from MD or $y_r = 0$ if not; μ is a normally distributed underlying mortality from MD and Φ is the cumulative normal distribution function. The probability of dying from MD can be seen to increase with μ. The mortality μ is modelled using a mixed linear model. For analyzing all data jointly, the model included random sire and dam effects accounting for relationships between sires and dams up to the base generation. The model is given as:

$$\mu_{ijr} = \alpha + s_i + d_j + e_{ijr} \qquad \text{(model 3)},$$

with μ_{ijr} the underlying mortality from MD in the chick r and α a general mean, s_i the random effect of the sire and d_j the random effect of the dam j, and e_{ijr} a random error.

Analysis was also done within lines, including MHC genotypes :

$$\mu_{ijklr} = \alpha + s_i + d_j + line_k + MHC_{kl} + e_{ijklr} \qquad \text{(model 4)},$$

where $line_k$ is the fixed effect of the line k (H, C, L), MHC_{kl} is the fixed effect of the MHC genotype l within the line k in the chick r, and all the other terms are as in model 3.

From the likelihood given above, a posterior distribution is constructed by adding a prior for sire and dam effects in order to make the effects random'. The resulting posterior can be maximized with respect to the unknown parameters using an iterative Newton raphson algorithm (Gianola and Foulley, 1983). For estimation of variance components, all fixed and random effects are integrated out from the posterior to obtain 'REML' estimates (Höschele et al., 1987). Because the model included all relationships between parents, the heritability is estimated in the base population. From model 4, 'MHC-free heritability' (Bentsen and Klemetsdal, 1991) were obtained.

Variance components and heritability were estimated the most appropriate way with a threshold model (see discussion). However, the threshold model procedure did not allow the calculation of significance of effects to be introduced in the model. Therefore, logistic regression was also used previously to estimate MHC effects. Moreover, MHC effects obtained from the logistic regression model 1 were in agreement with estimates from threshold model 4 (data not shown).

Genetic Correlation between Mortality from Marek's Disease and Antibody Response to SRBC

Pearson correlations between estimated breeding values (BV) for mortality from MD and Ab response to SRBC were calculated per sex within the H, C and L lines. Estimated BV for mortality from MD of ninth generation animals used to produced challenged chicks were obtained from model 3 (MD.BV) and model 4 (MDMHC.BV); MDMHC.BV are the "MHC-free estimated BV". Likewise, BV for Ab response to SRBC were estimated in the same ninth generation animals used to produced challenged chicks by using model 5 (Ab.BV) (Pinard et al., 1992a) and model 6 (AbMHC.BV) (Pinard et al., 1992b):

$$Ab_{mnr} = \delta + sex_m + G_n + U_{mnr} + e_{mnr} \qquad \text{(model 5)},$$

with δ a constant, sex_m the fixed effect of the sex, G_n the fixed effect of the generation, U_{mnr} the random additive genetic effect on the Ab response to SRBC in the chick r.

$$Ab_{klmnr} = \delta + line_k + MHC_{kl} + sex_m + G_n + U_{klmnr} + e_{klmnr} \qquad \text{(model 6)},$$

with all the terms as previously described. Model 5 was applied using all Ab data and relationships of the ten generations of selection. Model 6 was applied using all Ab and MHC data of the ninth and tenth generations and all relationships of the ten generations. Analysis was performed by PEST (Groeneveld, 1990; Groeneveld and Kovak, 1990), using an heritability of .31 for the Ab response as estimated on all selection data (Pinard et al., 1992a). Ordinary correlations (C) between estimated BV for traits A (EBV_A) and B (EBV_B) were shown to underestimate, in absolute value, the true genetic correlations (Blanchard et al., 1983). Under the realistic assumption of equal number of offspring per families and of uncorrelated errors due to measurement of the two traits on different animals, adjusted correlations (C_{adj}) can be obtained as following:

$$C_{adj} = \frac{1}{\sqrt{\dfrac{Var(EBV_A)\ Var(EBV_B)}{\sigma^2_{gA}\ \sigma^2_{gB}}}}\ C$$

with $Var(EBV_A)$ and $Var(EBV_B)$ the variances of EBV_A and EBV_B, respectively, and σ^2gA and σ^2gB the genetic variances of traits A and B, respectively.

RESULTS

Effects of selection on MD mortality

Gross examination of remaining birds at 17 wk of age did not show any sign of MD. The L line was significantly more susceptible than all the other groups as expressed by the crude OR (Table 1). By contrast, the H line was not significantly more resistant than the C line. There was no significant difference in mortality between the two F1 reciprocal crosses. The F1 showed positive heterosis for the resistance to MD.

TABLE 1. Mortality (%) from Marek's disease between five and 17 weeks and crude odds ratio (OR) in the high (H), control (C) and low (L) lines and in the F1.

Line	Mortality (%)[1]	OR[2]
H	40.9[A]	.92[A]
C	42.8[A]	1[A]
L	70.1[B]	3.13[B]
$H_{♂} \times L_{♀}$[3]	47.7[A]	1.22[A]
$L_{♂} \times H_{♀}$[3]	47.4[A]	1.21[A]
F1[4]	47.5[A]	1.21[A]

[1] % of mortality were compared by a chi-square test

[2] OR were calculated by taking the C line as reference and were compared by a chi-square test

[3] F1 reciprocal crosses

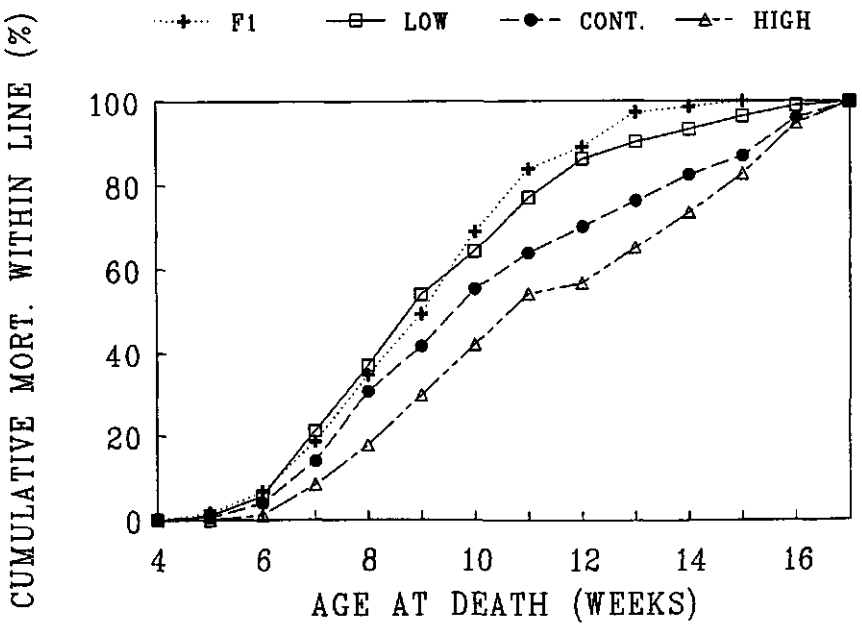
[4] Whole F1

[A,B] Different superscripts indicate differences ($P < .01$) between lines

Time of Death from Marek's Disease

There was no hatch effect on death by MD. Average wk of death from MD was 11.8, 10.8 and 9.7 in the H, C and L lines, respectively and was 9.5 in the F1. Cumulative mortality by MD for the different lines is shown in Figure 1. Survival analysis showed significant differences ($P<.01$) between the H, C and L lines for time of death, and the F1 was similar to the L line. The average wk of death of F1 chicks originated from high line hens died later than those from low line chicks (9.6 vs. 9.4) but the difference was not significant.

FIGURE 1. Cumulative mortality from Marek's disease in % of the total number of animals dead between five and 17 weeks of age in the high, control (cont.) and low lines and in the F1.



Effects of MHC types on Mortality from Marek's Disease

Number of animals per MHC genotype in the H, C and L lines and in the F1 are given in Table 2. In addition, two animals from the H line were typed as 119-124 but were removed from the analysis because of insufficient number. As a result of selection for Ab response to SRBC, all MHC haplotypes were not equally present in selected lines of the ninth generation, and the 121 B-haplotype was absent in the L line (Pinard et al., 1992b). Therefore, all ten genotypes could not be produced in the H and L lines and in F1.

TABLE 2. Number of animals per B-genotype in the high (H), control (C) and low (L) lines and in the F1

	Line			
Genotype	H	C	L	F1
114-114	7	54	75	72
114-119	0	32	52	96
114-121	31	34	0	116
114-124	0	39	42	67
119-119	32	66	27	47
119-121	24	28	0	66
119-124	0	59	42	59
121-121	76	54	0	0
121-124	21	35	0	71
124-124	15	57	36	37
ALL	206	458	274	631

The overall effect of MHC genotypes on MD mortality was the most significant in the C line (Table 3).

Genotype estimates in the C and in the H lines were similar, the 114-114, 114-124 and 124-124 genotypes showing the highest susceptibility. In the L line, there was little variation in genotype estimate, the 114-114 genotype showing significantly more susceptibility than all the other genotypes. In the F1, the 114-114 genotype showed also the highest susceptibility, but the ranking of genotype estimates was different from the other lines.

Estimates of MHC haplotype effects (expressed as risk, ß) in the C and L lines and in the F1 are shown in Table 4. In the H line, haplotype effects were not estimated because of missing genotypes. There was no significant difference between haplotypes in the L line. In the C line, the 124 haplotype was associated with the highest susceptibility, whereas the 121 and 119 haplotypes were showing the highest resistance. On the contrary, the 124 haplotype was associated with the highest resistance in F1.

TABLE 3. Estimates (ß) of B-genotype (Geno) effect, using logistic regression model 1[1] within lines, on mortality from Marek's disease between five and 17 weeks in the high (H), control (C) and low (L) lines and in the F1[2,3]

Line							
H		C		L		F1	
Geno	ß	Geno	ß	Geno	ß	Geno	ß
114-114	0^{abc}	114-124	$.29^{a}$	114-114	0^{a}	114-114	0^{a}
124-124	$-.15^{a}$	124-124	$.13^{a}$	119-124	-1.26^{b}	114-121	$-.11^{a}$
119-121	$-.58^{ab}$	114-114	0^{ab}	119-119	-1.48^{b}	114-119	$-.19^{a}$
114-121	$-.68^{abc}$	119-121	$-.35^{abc}$	114-124	-1.50^{b}	119-121	$-.22^{ab}$
121-121	-1.35^{abc}	121-124	$-.96^{bcd}$	124-124	-1.52^{b}	114-124	$-.28^{ab}$
121-124	-1.78^{bc}	119-124	$-.97^{cd}$	114-119	-1.60^{b}	124-124	$-.40^{ab}$
119-119	-1.81^{c}	114-121	-1.00^{cd}			119-119	$-.48^{ab}$
		119-119	-1.23^{cd}			119-124	$-.50^{ab}$
		121-121	-1.30^{cd}			121-124	-1.11^{b}
		114-119	-1.56^{d}				
P[4]	.091		.004		.010		.049

[1] log $Odds_{ilr} = \tau + s_i + MHC_l + e_{ilr}$ with s a constant, MHC_l the fixed effect of the MHC genotype l in the chick n, s_i the random effect of the father i of the chick r, and e_{ilr} a random error.
[2] Genotypes are ordered from the most susceptible to the most resistant within lines.
[3] The 114-114 genotype was taken as a reference.
[4] P is the significance of the B-genotype effect within lines.
^{a,b,c,d}: Estimates with different superscripts indicate differences ($P<.05$) between genotypes within lines.

TABLE 4. Estimates (ß) of B-haplotype effect, using logistic regression model 2[1] within lines, on the mortality from Marek's disease between five and 17 weeks in the control (C) and low (L) lines and in the F1[2]

Line					
C		L		F1	
Haplotype	ß	Haplotype	ß	Haplotype	ß
124	.23[a]	114	0[a]	114	0[a]
114	0[ab]	124	-.40[a]	119	-.13[ab]
121	-.58[b]	119	-.41[a]	121	-.31[ab]
119	-.74[b]			124	-.57[b]

[1] $\text{log Odds}_{ilr} = \tau + s_i + \Sigma_l \text{Haplo}_l + e_{ilr}$, with τ a constant, s_i the random effect of the father i of the chick r, Haplo_l the fixed effect of the MHC haplotype l (present or absent) in the chick r, and e_{ilr} a random error.
[2] The 114 haplotype was taken as a reference.
[a,b]: Estimates with different superscripts indicate differences (P<.05) between haplotypes within lines.

Heritability and 'MHC-free heritability' of Resistance to Marek's Disease

Heritability estimates within lines and overall lines are shown in Table 5. Heritability estimated overall lines by taking into account all relationships was .40. Heritabilities estimated within lines were higher, especially in the L line. The 'MHC-free heritability' was lower than the heritability in the C line but the inverse was observed for selected lines.

TABLE 5. Heritability and 'MHC-free heritability' estimates on underlying scale, using threshold models 3[1] and 4[2], of mortality from Marek's disease between five and 17 weeks in the high (H), control (C) and low (L) lines, and overall lines

	Line			
	H	C	L	Overall
h^2	.45	.51	.78	.40
$h^2_{MHC\text{-}free}$	.57	.48	.83	.60

[1] $\mu_{ijr} = \alpha + s_i + d_j + e_{ijr}$ [2] $\mu_{ijklr} = \alpha + s_i + d_j + \text{line}_k + \text{MHC}_{kl} + e_{ijklr}$
with μ_{ijr} and μ_{ijklr} the underlying mortality from MD in the chick r, α a general mean, s_i the random effect of the sire and d_j the random effect of the dam j, line_k the fixed effect of the line k, MHC_{kl} the fixed effect of the MHC l within the line k, and e_{ijr} and e_{ijklr} a random error.

Genetic Correlation between Mortality from Marek's Disease and Antibody Response to SRBC

Estimated BV for mortality from MD (MD.BV) in relation to estimated BV for Ab response to SRBC (Ab.BV) are shown in Figure 2 and average values are presented in Table 6. Whereas the H, C and L lines were significantly different genetic groups for Ab response to SRBC, only the L line had a significantly higher estimated mean BV for mortality from MD than the C and H lines.

FIGURE 2. Estimated breeding values for mortality from Marek's disease (MD.BV) using model 3, in relation to estimated breeding values for antibody response to sheep red blood cells (Ab.BV) using model 5, in the high (H), control (C) and low (L) lines.

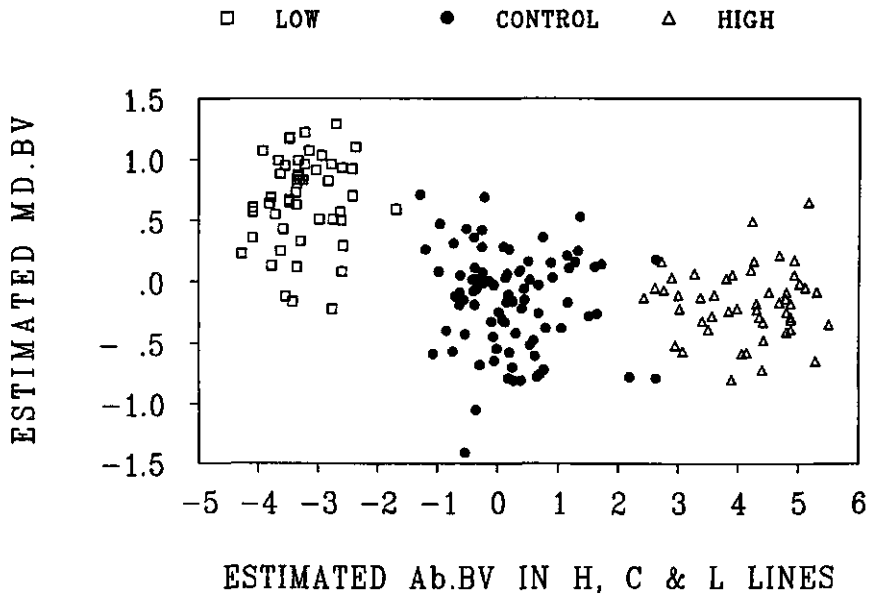


TABLE 6. Mean estimated breeding values (±SD), for mortality from Marek's disease (MD.BV) using model 3[1], and for antibody response to sheep red blood cells (Ab.BV) using model 5[2], in the high (H), control (C) and low (L) lines

	Line		
	H	C	L
n[3]	50	85	51
MD.BV	-.19±.28^{A}	-.16±.41^{A}	.67±.37^{B}
Ab.BV	4.13±.82^{A}	.23±.82^{B}	-3.25±.52^{C}

[1] $\mu_{ijr} = \alpha + s_i + d_j + e_{ijr}$, with μ_{ijr} the underlying mortality to MD in the chick r, a a general mean, s_i the random effect of the sire and d_j the random effect of the dam j, and e_{ijr} a random error.
[2] $Ab_{mnr} = \delta + sex_m + G_n + U_{mnr} + e_{mnr}$, with Ab_{mnr} the Ab titer in the chick r, δ a constant, sex_m the fixed effect of the sex, G_n the fixed effect of the generation, U_{mnr} the random additive genetic effect on the Ab response to SRBC in the chick r, and e_{mnr} a random error.
[3] n is the number of animals of which breeding values were estimated
^{A,B,C} indicate significant differences (P<.001) in mean MD.BV or Ab.BV between lines

Correlation between estimated BV for mortality from MD and for Ab response were lower in females than in males, especially in the H line (Table 7). Correcting BV for MHC effects did not affect the correlations in the C line. On the contrary, correlation between estimated BV for mortality from MD (MD.BV and MDMHC.BV) were lower with 'MHC-free' estimated BV for Ab response (AbMHC.BV) than with estimated BV for Ab response (Ab.BV) in the L line. The inverse was observed in H line hens whereas correlation between estimated BV for Ab response (Ab.BV and AbMHC.BV) were lower with 'MHC-free' estimated BV for mortality from MD (MDMHC.BV) than with estimated BV for mortality from MD in the H line cocks. This results tended to indicate a positive effect of MHC genotypes on the relationships between Ab response and mortality from MD in the L line and in the H line cocks, and a negative effect in the H line hens. When comparing MHC effects on Ab response and on MD mortality, the most susceptible genotypes tended to have higher Ab response in the H line (Figure 3a); no clear relationship could be defined in the L and C lines (Figures 3a and 3b).

FIGURE 3. Estimated effects of B-genotypes on mortality from Marek's disease (MD) using model 1, in relation to their estimated effects on antibody (Ab) response to sheep red blood cells using model 6, in the high (H) and low (L) lines (Figure 3a), and in the C line (Figure 3b). Some genotypes were not considered in the H line because they were present in less than 3% of the animals.

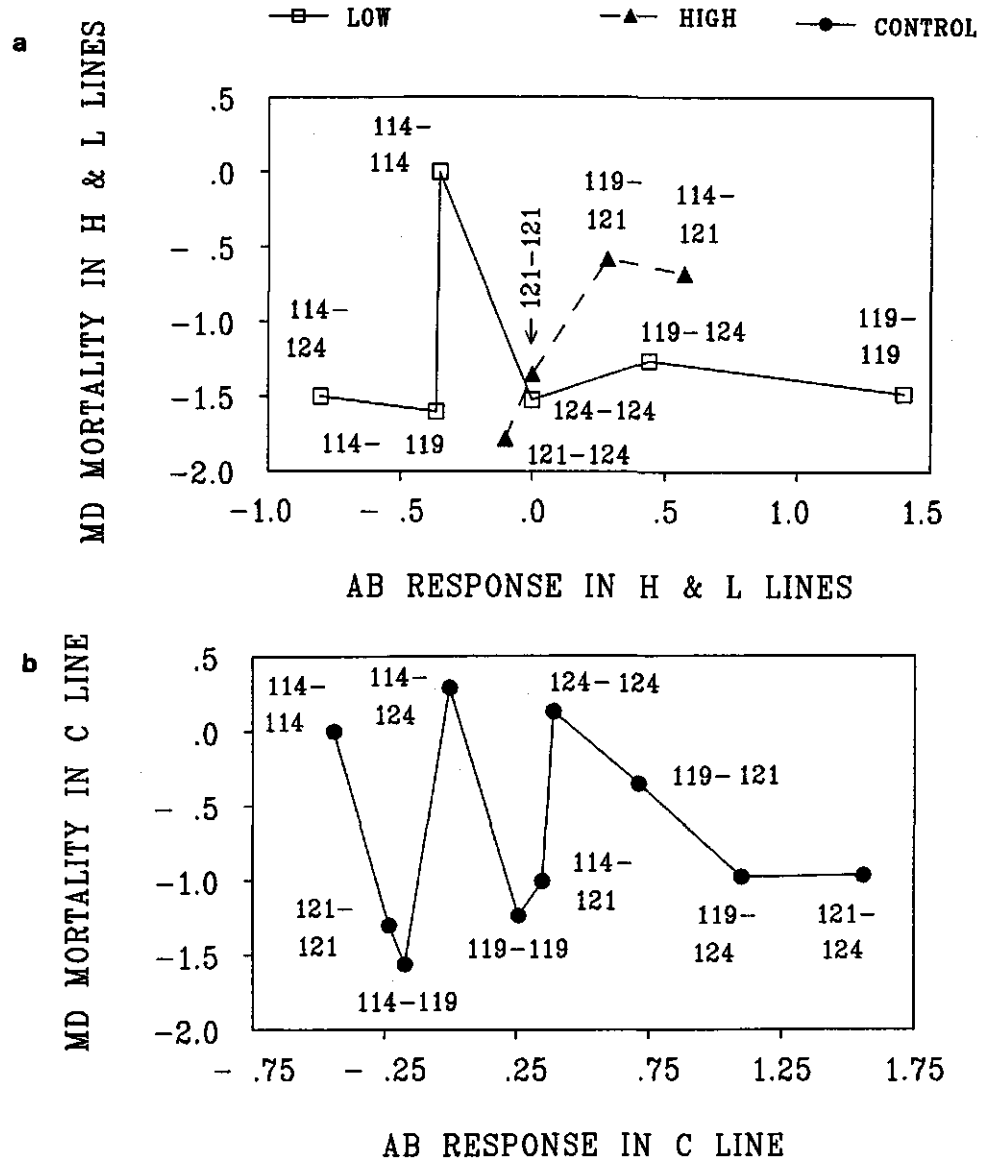


TABLE 7. Correlations (C) and adjusted correlations (C_{adj}) between estimated breeding values for mortality from Marek's disease using models 3[1] (MD.BV) and 4 (MDMHC.BV)[2], and for antibody response to sheep red blood cells using models 5[3] (Ab.BV) and 6[4] (AbMHC.BV), per sex in the high (H), control (C) and low (L) lines

		Correlation							
		C				C_{adj}			
		Ab.BV		AbMHC.BV		Ab.BV		AbMHC.BV	
	Line	hen	cock	hen	cock	hen	cock	hen	cock
MD.BV	*H*	-.23^{o}	.34^{o}	-.05	.36^{o}	-.41	.34	-.21	.42
	C	-.13	-.03	-.12	-.05	-.30	-.07	-.30	-.18
	L	.08	.13	.01	-.02	.37	.40	.02	-.05
MDMHC.BV	H	-.30^{+}	.02	-.07	.08	-.60	.50	-.18	.18
	C	-.08	-.01	-.13	-.07	-.16	-.02	-.28	-.14
	L	.07	.22	-.09	-.02	.25	.52	-.27	-.05

[1] $\mu_{ijr} = \alpha + s_i + d_j + e_{ijr}$
[2] $\mu_{ijklr} = \alpha + s_i + d_j + line_k + MHC_{kl} + e_{ijklr}$
[3] $Ab_{mnr} = \delta + sex_m + G_n + U_{mnr} + e_{mnr}$
[4] $Ab_{klmnr} = \delta + line_k + MHC_{kl} + sex_m + G_n + U_{klmnr} + e_{klmnr}$
with μ the underlying mortality from MD in the chick r, α a general mean, s_i the random effect of the sire and d_j the random effect of the dam j, $line_k$ the fixed effect of the line k, MHC_{kl} the fixed effect of the MHC l within the line k, Ab the Ab titer in the chick r, δ a constant, sex_m the fixed effect of the sex, G_n the fixed effect of the generation, U_{mnr} the random additive genetic effect on the Ab response to SRBC in the chick r, and e a random error.
$^{+}$ P<.10 ^{o} P<.20

DISCUSSION

Resistance to Marek's disease infection depends on several factors such as age at exposure, presence of maternal antibodies, virulence of the virus strain, genetic constitution (Calnek, 1986).

Infection at day-old by contact with spreader chicks was chosen to reproduce natural infection conditions and because most exposures to the virus are occurring soon after hatching (Calnek, 1986). At this stage, effect of maternal antibodies on the outcome

of the disease might be expected. There was no history of MD infections in the lines used in this study, but all antecedents were vaccinated against MD and it was not measured whether H line hens produced more antibodies against MD vaccine than C and L hens. Lee and Witter (1991) showed that passive antibodies against MD virus delayed the disease process but did not prevent death. Maternal antibodies may act in neutralizing free virus or in coating the surface of lymphocytes preventing their cytolysis (Sharma, 1988). However, time of death is also influenced by genetic constitution: in the F1, chicks from H line hens died slightly later than chicks from L line hens, but the F1 died at about the same time as the L line.

As we showed that selection for high and low Ab response to SRBC lead to an asymmetric correlated response in resistance to MD, results of other similar experiments, which did not include a control line, stating an improvement of disease resistance should be looked at with caution (e.g. Dunnington et al., 1986). Asymmetric correlated response in resistance to MD may be due to several reasons:

(i) the level of resistance for the virus strain used was already high in the base population, so that the resistance could not be highly improved upwards. This assumption could be verified by trying to select the H line for higher resistance to the MD virus used.

(ii) during selection, genetic potential for selecting downwards for resistance to MD became higher in the L line than the one for selecting upwards in the H line. In other words, frequency of one or more loci associated with resistance to MD was relatively high in the base population and was brought downwards to intermediate frequencies, leading to increase of heritability. Indeed the heritability estimate of resistance to MD was the highest in the L line. Dramatic increase of heritability in the L line may indicate the role of a few loci only. Heritability and genetic correlation may vary in relation to selection applied and to gene frequency changes (Falconer, 1989), and the lines differ for at least MHC genes and other immune response genes (Pinard et al., 1992b).

(iii) genes favoured by selection in the H responders line were not associated with an enhanced resistance to MD, whereas some genes selected in the L line had potentially a direct or linked negative effect on resistance to MD. This assumption may be illustrated by the 114-114 genotype which became the most present genotype in the L line (44.6% in the tenth generation, Pinard et al., 1992b) and was shown to be associated with a relatively low Ab response and the highest relative susceptibility to MD (Figure 3a).

Assumption (ii) explains in fact assumption (i) from a genetic standpoint, and may be also directly related to assumption (iii). Assumption (iii) could be confirmed by the combination of a positive and negative correlation between mortality from MD and Ab response to SRBC found in H line cocks and hens, respectively (Table 7), leading to intermediate values, i.e., no genetic progress, for the progeny.

Selection for Ab response may have affected the estimation of genetic parameters and of MHC effects at different levels. Under selection, covariance between single gene effects and polygenic effects have been shown to be negative (Kennedy et al., 1992). Although mortality from MD was not directly the trait selected against, this phenomenon may have occurred for both Ab response and mortality from MD, and would explain the higher values of 'MHC-free heritability' estimates as compared to the heritability in the selected lines (Table 5). Likewise, this phenomenon may explain partly the positive effects of MHC on correlations between Ab response and mortality from MD found mostly in the selected lines; however, possible different MHC effects on both traits and truly negative interaction between MHC effects and polygenes may be still considered. Finally, estimates of MHC effects on both traits in the selected lines may biased when data on previous generations are not known (Kennedy et al., 1992).

It is clear anyway that MHC (or MHC-linked) genes as well as non-MHC effects are important in the genetic control of resistance although the underlying mechanisms are not yet understood. Highest resistance to MD may be obtained by specific haplotype combinations in given line crosses, due to genetic complementation within and outside of the MHC (Briles et al., 1982; Schierman and Collins, 1987; Steadham et al., 1987). Therefore, estimated haplotype effects can only give an idea of the average effect of the haplotype. Direct effect of MHC in resistance to MD may involve an active rejection of MD transformed cells (Longenecker et al., 1976), and class I restriction of cytotoxic T cells may play an important role in immunity to MD (Schierman and Collins, 1987). In White Leghorns, resistance and susceptibility to MD was often associated with the B^{21} and B^{19} haplotypes, respectively (Bacon, 1987). No direct comparison can be made with our study because none of the haplotypes were identical for both B-G and B-F with reference B haplotypes; for example, B^{121} haplotype showed similarities with B^{21} for B-F but not for B-G, whereas B^{119} showed similarities with B^{19} for B-G but not for B-F (Pinard and Hepkema, 1992).

For analyzing disease data, an epidemiological approach was successfully applied and unlarged to genetic analysis. Mortality from MD and effects of MHC in chickens have been traditionally assessed by comparing with a chi-square test pairs of genetic stocks or MHC types. By contrast, logistic regression could estimate simultaneously effects of more than two classes when taking into account relationships between individuals, which is crucial since disease trait is influenced by both MHC and non-MHC genes.

Heritability estimates of resistance to MD from .06 to .67 have been reported (reviewed by Gavora, 1990). But to our knowledge, no heritability value of resistance to MD estimated with a threshold model had been reported yet. The threshold model corresponds to the genetic concept for discrete traits under polygenic control as postulated by Wright (1934). On various points, the threshold model is more realistic than the logit model as it uses a binomial expression on the observed scale, and postulates an underlying normally distributed liability, which corresponds well to the general genetic assumption of normally distributed effects. Moreover, compared to linear models used for discrete data, the threshold model has the crucial advantage in this study that heritability estimates are indifferent to the occurrence of the disease.

We failed to establish a clear relationship between resistance to MD and Ab production to SRBC. Likewise, although cell-mediated immunity may be important in MD (Schat, 1991), no general correlations between different measures of cell-mediated immunity and resistance to MD could be established either (Calnek et al., 1989; Gavora et al., 1990). Indirect selection for resistance to MD by selecting for an unique immune parameter, which would not require exposure to the disease, does not seem to be feasible. Because of the complexity of mechanisms involved, research on relationship between resistance to MD and simultaneous combination of different immune parameters should be performed.

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GENERAL DISCUSSION

GENERAL DISCUSSION

The main goal to be achieved in this study was to assess the relationships between antibody (Ab) response to sheep red blood cells (SRBC), resistance to Marek's disease (MD) and effects of the major histocompatibility complex (MHC) (Figure 1). Results obtained and methodology used, as well as prospective research will be discussed herein in the broader view of breeding for disease resistance.

STRATEGY FOR BREEDING FOR DISEASE RESISTANCE

Main steps in breeding for disease resistance may be summarized as follows:

-1- Define the disease resistance trait (resistance to infection, to tumor development, to death...).

-2- Establish the heritability of the disease trait, the environmental factors (genetic and epidemiological analysis).

-3- Search for marker, single genes associated with the disease trait and estimate effects on the trait.

-4- Search for traits (i.e. combination of immune parameters) correlated with the disease trait.

-5- Estimate heritability and genetic correlations (if multiple immune parameters) of (4), effects of (3) on (4), and genetic correlations between (4) and the disease trait.

-6- Search for possible antagonisms between (1), (3) or (4) and economically important traits.

-7- If (3), (4) and (5) are promising, use separately or combined, using e.g. (multitrait) selection, transgenesis, marker assisted selection. If none of them is possible then select directly on disease trait. In both case, take account of (6), by e.g. combining production trait in selection index.

-8- Estimate again regularly the genetic level of the population for the different traits, the heritabilities and genetic correlations, which may vary under selection and gene frequency changes.

-9- And always be aware that the results of the study may be valid for a given genetic stock only.

The approach of the present study was chronologically different, since research focused firstly on the putative correlated immune trait (Ab response to SRBC) and its genetic control including the effect of single genes (MHC). Genetic control of disease trait (MD) and relationships between the two traits and single genes were subsequently analyzed.

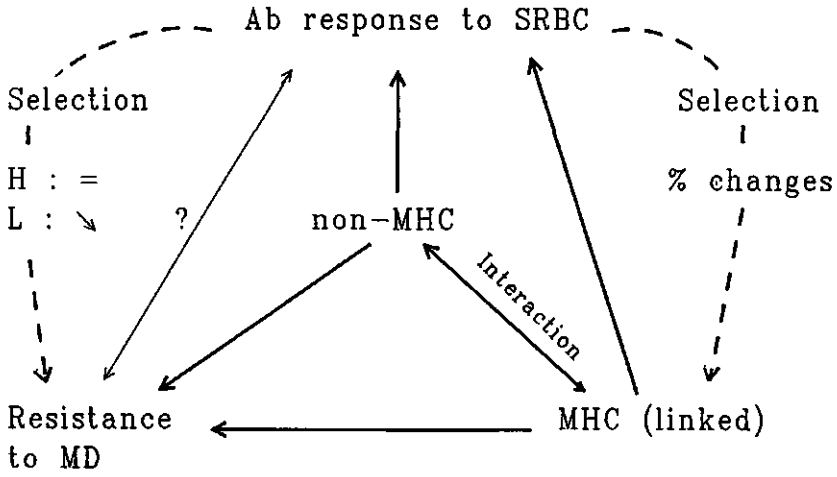

FIGURE 1. Schematic representation of the genetic relationships established in this thesis between Ab response to SRBC, resistance to MD and effects of MHC (linked) and non-MHC genes. Dotted arrows indicate effects of selection for Ab response to SRBC on (i) changes in MHC type frequency in the selected lines, and on (ii) decrease and absence of increase of resistance for MD in the L and H lines, respectively. Full arrows indicate effects of MHC (linked) and non MHC genes, and "interaction" indicate pleiotropic effects of non MHC genes. Genetic relationship between Ab response to SRBC and resistance to MD was unclear ("?").

GENETIC CONTROL OF AB RESPONSE TO SRBC AND OF RESISTANCE TO MD

MHC and non-MHC control

Antibody response to SRBC and resistance to MD showed to have a high heritability in common, .31 and .40 in the base population, respectively (Chapters 1 and 5), and to be controlled by MHC (linked) genes and non-MHC polygenes (Chapters 3-5; Figure 1). From the variance analysis, MHC genotypes showed to contribute 2.5% of the total variance of Ab response (Chapter 4), and 3% of the total variance of mortality from MD (difference between heritability and MHC-free heritability in the C line, Chapter 5). In other words, maximum improvement of immune response and resistance to MD may not be achieved by selection on MHC only, but rather on the combination of MHC and other polygenes. For both traits, MHC may have a direct effect although the analysis could not distinguish between direct and closely linked effects.

Identification of MHC haplotypes vs. large scale typing

In chapter 2, we used biochemistry to characterize B-G and B-F antigens. By contrast, for large scale typing in genetic analyses (Chapters 3, 4 and 5), serological MHC typing was preferred to biochemistry because of the simplicity of the hemagglutination test. Moreover, no recombination has ever been found yet between B-F and B-L genes (Hála et al., 1988) and biochemical analysis did not reveal here any recombination between B-F and B-G alleles (Chapter 2). Therefore, serological typing, which detects mainly B-G variants (Guillemot and Auffray, 1990), likely detected in fact here a series of unique haplotypes differing for all three regions. In other words, the results of this study are not in contradiction with the assignment of resistance to MD to the B-F/B-L region rather than to the B-G region (Briles et al., 1983; Blankert et al., 1990b), and with the B-L restriction of cellular interactions important for Ab production (Bacon and Dietert, 1991).

Different genetic control between H and L lines ?

For both Ab response to SRBC and resistance to MD, the question arises whether H and L response, or resistance and susceptibility are different traits, or in other words are controlled by different set of genes. Kinetics of Ab response to SRBC (Kreukniet and Van der Zijpp, 1990) as well as of resistance to MD (Chapter 5) differed between the H and L lines. When estimating the heritability in the H and L lines separately, we found in both cases, although the difference was more striking as far as resistance to MD was concerned, a higher heritability in the L line (Chapters 1 and 5). In lines of mouse selected for high and low Ab production to SRBC, Mouton et al. (1979) found that the number of loci controlling Ab response to SRBC was higher in case of immunization with an optimal dose than with a threshold dose. In parallel, Kreukniet and Van der Zijpp (1990) showed in previous generation of this study that a dose of 1 ml of 25 % SRBC induced after five days (selection criterium) an optimal response in the L line but not in the H line where an optimal response was obtained with a higher dose. Thus, for a given dose, the number or expression of loci involved in the response to SRBC may differ between lines. Immunological mechanisms (antigen processing, macrophage activity, T cell activity...) might also differ in quality and relative importance between H and L animals, explaining the difference in responsible genes.

RELATIONSHIP BETWEEN AB RESPONSE TO SRBC, MHC AND RESISTANCE TO MD

The relationship between Ab response to SRBC and resistance to MD could be less clarified than the individual genetic control of the two traits (Figure 1). However, some outlines may be drawn from the present results (Chapter 5) and may be discussed in relation to the immunological characteristics of the lines.

Genetic correlation between Ab response to SRBC and resistance to MD

Two results were in favour of a positive relationship between Ab response to SRBC

and resistance to MD: genetic correlations between the two traits were positive in the control line, and a correlated response was observed in the L responders line. By contrast, the fact that H line animals were not significantly more resistant to MD than the C line chicks seems to contradict the assumption of a positive relationship. More generally, we may wonder which kind of experiment is ideal to assess the relationship between two traits. In the present study, more important correlated response downwards resulted likely from a low frequency of genes associated with higher susceptibility to MD in the base population. Unfortunately, until these genes are identified, there is no way to prove this assumption. Asymmetric correlated response may also result from directional dominance, natural selection opposing one direction but not hindering the other one or negative effect of inbreeding (Falconer, 1989). But the latter effects were likely not present here. Therefore definite conclusions regarding the possibility or impossibility of using a given trait (e.g. Ab response to SRBC) to improve disease resistance (e.g. to MD) should not be drawn from the correlated response in one direction only, because results of the experiment clearly depend on the gene frequency in the base population. The other explanation for this asymmetry would be that genes having a negative effect on both Ab response to SRBC and MD are more numerous than those having a positive effect on both traits. This assumption may be strengthened by the previous hypothesis of different genetic control between the H and L lines. In this case, selection for Ab response to improve genetic resistance would not be applicable. Understanding of the correlation between the two traits was even made more complex by a possible genotype by sex interaction. A more powerful way of assessing the relationship between the two traits would be a multivariate analysis in a random background population such as an enlarged control line, or a F2, and including data on both sexes. Moreover, this multivariate analysis should be able to combine a continuous trait and a threshold trait.

Correlated effects of MHC on Ab response to SRBC and resistance to MD

Whereas changes in MHC types distribution obviously contributed to the response to selection for H and L Ab response to SRBC, MHC contribution in favour of or against correlated response for resistance to MD effect was not clearly shown. In the C line, free from selection interferences, no relationship appeared between MHC effects on Ab

response to SRBC and effects on resistance to MD (Chapter 5, Figure 3b). From this Figure, if only the 114-124 and 121-124 B-genotypes had been present for example, we would have concluded on a positive correlated effect. Therefore, such conclusion obviously depend on the genotypes displayed in the population. And, as said previously, correlated response to selection may be accelerated, or on the contrary refrained, depending this time, on the alleles present. These considerations are valid also for all other alleles than MHC alleles not identified here, which have a significant effect on both traits.

Immunological differences between the H and L lines

Could a correlated response for resistance to MD be expected from selection for Ab response? The major components of the immune system (Ab response, phagocytosis, cell-mediated immunity) may be under different genetic control in mice (Biozzi et al., 1982), and negative correlations were shown between phagocytosis and cell-mediated immunity in chickens (Cheng and Lamont, 1988; Cheng et al., 1991). In mice, selection for Ab response to SRBC did not modify T-cell mediated immunity but affected macrophage activity; the catabolic rate of macrophage was higher in the low line, leading to less efficient antigen processing and handling to B-cells. In the present experiment, no other immunological differences except Ab response to SRBC and other antigens could be found after five to seven generations of selection between the H and L lines in terms of macrophage activity as measured by carbon clearance and carrageenan injection, or cell-mediated immunity as measured by in vitro stimulation test to PHA and ConA or in vivo with PHA (Van der Zijpp and Nieuwland, 1986; Van der Zijpp et al., 1988, 1989). Only more immunocompetent cells were detected in H line spleen than in L line spleen (Donker, 1989). Recently, in vivo T-cell reactivity was measured by DTH (delayed-type hypersensitivity) response to several T-cell dependent antigens; the L line responded significantly less than the C line, whereas the H line did not respond higher than the C line. (Parmentier et al. 1992, unpublished results). It would be of course very tempting to correlate this asymmetry with the results regarding resistance to MD. In parallel, Siegel et al. (1992) found a higher B^+ cell percentage at the expense of T cells in the H line spleen and blood as well as a higher $CD4^+/CD8^+$ ratio in the H line spleen than in the L line spleen. Unfortunately, the C line was not tested. CD4 and CD8 are accessory

molecules for class II and class I MHC antigen-restricted T cells, respectively, and modulate the immune response (Vainio and Lassila, 1989). Moreover, most $CD4^+$ cells are T-helper cells, whereas most $CD8^+$ cells are cytotoxic T cells and MHC-restriction of cytotoxic T cells may be an important mechanism in immunity to MD (Schierman and Collins, 1987). Interestingly, Hála reported a higher $CD4^+/CD8^+$ ratio in resistant line of chickens to Rous sarcoma virus than in susceptible ones. But resistance or susceptibility to MD involve a series of complex mechanisms from contact with the virus, infection, development of the pathogenesis leading finally to recovery or death. It is therefore difficult, and even impossible to relate resistance to MD to a single mechanism or parameter. MD pathogenis may be divided in four overlapping phases: cytolytic infection, latent infection, transformation and immunosuppression (Calnek, 1986). B cells were reported as the main target cells for early cytolytic infection (Schat, 1991). Thus, it may be speculated that a higher B cell percentage is a disadvantage at this stage for H line animals as compared to L line chicks. But, as a result, T cells are activated and initiate the immune response. The higher T cell reactivity in H line animals than in L line might then play an important role. However, in the transformation phase, T cells become also susceptible to transformation (Schat, 1991). Although cell mediated immunity may be important in the outcome of the disease, nature of cell mediated responses against MD viral antigens are likely different than activated responses against tumor antigens (Schat, 1991). This may explain why pairs of chicken lines selected for different immunological parameters differed for resistance to MD tumor cell line but not for resistance to MD which involves more complex mechanisms (Yamamoto et al., 1991). Obviously, further fundamental research is needed to clarify all the mechanisms involved during MD infection. In parallel, it would be very interesting to conduct a multitrait analysis combining Ab response to SRBC, resistance to MD, DTH response, MHC types and T-cell markers to assess whether the apparent immunological differences observed between the lines are fortuitous or not.

IMPORTANCE OF THE METHODOLOGY IN THE ANALYSIS

The choice of methodology to analyze a trait depends essentially on the type of data and design, the nature of trait and the effects which can modify the trait. Most studies on effect of MHC on different traits in chickens so far were based on more simple designs than our study. Four main problems had to be taken into account here: two traits combining polygenic inheritance and single gene effect (Ab response to SRBC and resistance to MD), a non-random distribution of single gene effects (selection data), environmental influences, and a binomial trait (resistance to MD).

Animal mixed model

The use of a mixed model, taking single gene effect and environmental effect (e.g., year effect) as fixed, and sire and dam effects as random was a minimum requirement. In related and, moreover, in selected animals which shared the same MHC genotype, background genes are not randomly distributed. Ignoring this non-random distribution would lead to very biased estimates of MHC effects and will incorrectly inflate the significance of these effects (Kennedy et al., 1992). The best way to overcome this problem is to take into account the complete relationship matrix and all generation data in the analysis. If single gene information is not known from the base population, the bias in the estimation cannot be completely eliminated (Chapters 3 and 5). In the present selection experiment, and likely in other experiments, typing techniques for single genes were not developed yet or not considered before knowing whether the selection would be successful or not. Considering the progress of molecular biology nowadays, it would be very advisable to keep frozen DNA samples from all individuals from the initiation of a selection onwards, and certainly from the base population if a control line is not kept. This would leave the possibility, if needed, to use later the single gene information.

Genotype model vs. haplotype model

Although results of genotype effects are more difficult to compare with other results than haplotype effects, especially when there are numerous genotypes, a genotype model

should be preferred upon a haplotype model when there is no identical heterozygote effect for every combination. And this can be tested only if all combinations are available. According to our data, the expectation, often widespread, of a general advantage or disadvantage of heterozygous status should be tempered. In the F2 where the design was perfectly balanced, no general heterozygous effect on Ab response was shown, whereas two combinations showed significant but opposite effects. In this case a haplotype model including a general heterozygote effect would be incorrect. Furthermore, it seems dangerous to anticipate on the value of missing genotypes from such a model without prior knowledge, as proposed by Bentsen and Klemetsdal (1991). Specific heterozygous advantage may result from specific gene complementation at one or several loci, and expressed as e.g. complementary molecule conformation important in the given antigen recognition and cell cooperation.

Threshold trait

Finally, two approaches were used to analyze the mortality to MD as a binary trait, a multivariate logistic regression and a threshold model. Both methods represent a great advance compared to chi-square methods more commonly performed. The latter allow only pairwise comparison and no simultaneous estimation of different effects such as multiple genotypes, and cannot correct for random parental effects. In some cases, the threshold model may have several advantages: (i) All the relationships between individuals can be taken into account. Family information would be crucial if several generations of data were used. (ii) The underlying normal distribution fits more the assumption of polygenic control, and heritability could be estimated which was independent from the incidence of MD. (iii) The model may be extended to any multinomial trait with more than two classes (e.g. lesions score, immunological test resulting in 3 or 4 classes such as titers...).

VALUES AND LIMITS OF A CONTROL LINE AND OF A F2

Usefulness of keeping a C line was proven at several steps of this study. For estimating the response to selection (Chapter 1), the C line was used to correct for large environmental effects when using the regression of cumulative response on cumulative selection differential, and it contributed to a more precise estimation of genetic parameter, and of fixed and random effects when applying the animal model. The C line could reflect the frequency of MHC types as they were likely in the base population and strengthen the assumption of a change in MHC type distribution (Chapter 3).
The C line was used as a reference to be compared with for MHC effect and resistance to MD in the selected lines, and showed in this very case the absence of upwards correlated response which could not have been shown otherwise (Chapters 3 and 5). However, it could be somewhat surprising that MHC effects in the C line differed from estimates in the F2, although both groups are in theory displaying a random background. This background was, however, not identical and MHC effect might depend on the genetic background. Furthermore, two generations of random matings to produce the F2 may have not been sufficient to break up all linkage disequilibria resulting from selection. Finally, a C line may be valuable only if inbreeding stays low, or in other words, if the number of effective parents is sufficient to ensure stability and minimum drift in terms of long-term selection (Gowe and Fairfull, 1990).

GENETIC EPIDEMIOLOGICAL APPROACH

The advantages of conducting a MD challenge as described (Chapter 5) were as follows: birds were produced according to chosen parental combinations and could be MHC typed; conditions were controlled and made identical for all birds; the nature of the virus was known. This sort of challenge was designed to answer precise questions regarding the genetic control of resistance to MD for animals in a specific habitat or ecosystem. However, more factors are interacting during 'real' infections, such as the age of animal, the presence of other diseases which predispose for further infection (e.g. MD and coccidiosis [Hartmann, 1987]), previous vaccination and medication, nutrition, density,

light, temperature, the farm as a general management effect, the region... Some of these factors are usually taken into account in the framework of a epidemiological study (e.g. Henken et al., 1992). By contrast, genetic effects (besides breed) are not commonly considered then. As we showed their importance and the possibility to include these genetic effects in a epidemiological multitrait analysis, both genetic and 'environment' factors could be simultaneously analyzed from farm data. Including reproduction and production data would even be an advanced step in disease control related to an economically important trait. But this control would require a closed cooperation between the different actors in the field. Moreover, if family information may be obtained, typing for genetic markers such as MHC could not be performed for all production chicks, but may be done on parents from initial stocks. Such an information network has been already established in Nordic dairy cattle breeding where veterinary and production data are gathered in breeding programmes with the cooperation of artificial insemination centres (see e.g. Solbu and Lie, 1990).

PROSPECTIVE APPLICATION OF ENHANCED IMMUNE RESPONSE AND OF MHC (OR OTHER GENES) FOR IMPROVING DISEASE RESISTANCE

Improving vaccination efficiency

Variability in binding capacity of MHC molecules to antigenic peptides may be also true in case of peptide vaccine, so that some animals may not be able to mount an efficient response to a given vaccine. Understanding of this mechanisms will help to produce more effective vaccines (Hoffman, 1992).

Improving genetic disease resistance

Possible applications in terms of improved genetic resistance depend on the structure of breeding and industry. In poultry breeding, few animals are used as breeding stock so that genetic improvement is mostly separated from production. Therefore,

improving disease resistance in poultry breeding would require only a selection of a limited number of animals which would diffuse the progress in all production animals. Gavora et al. (1986) succeeded in improving simultaneously egg production and resistance to MD in a White Leghorn line. However, indirect selection based on correlated traits such as simple immune parameters or on single genes such as MHC genes would not require exposure to the disease of challenge animals which would be lost for the production, and therefore would be a better alternative. Selection on single genes would have the further advantage of being possible at a very early age, i.e. before the desired phenotype is expressed.

Use of favourable alleles

As shown by many experiments (e.g. Hartmann, 1987; Blankert et al., 1990a), effect of MHC on the resistance to MD depended on the genetic background. Especially the F1 showed a different profile for MHC effects than in the other lines (Chapter 5). But this possible variability in effect should not be considered as an obstacle to practical application of MHC in improving disease resistance. This implies only a preliminary study, which should be performed anyway, of MHC effect in the population of interest (line or line cross), to determine the most favourable alleles. Once the favourable MHC alleles and the interesting immune traits have been defined, they can be used separately each, i.e. selecting alleles or introducing them by introgression or transgenesis when the technique is available on one hand, and selecting for immune traits on the other hand, or they can be combined in marker assisted selection (MAS) (Smith and McMillan, 1989). The efficiency gained from MAS will be high if the heritability of the trait of interest is low and the additive variance due to the single gene high.

The question may still remain whether to, e.g., select the most favourable alleles among those already present in the population, or to introduce other alleles which may have potentially an even higher effect but whose effect is not known in the given population. The answer likely depends in the future on the technical possibility to introduce a new gene in sufficient numbers of animals. Finally, the possibility of increasing

the number of MHC gene copies and the related dose-dependant of gene product (Bloom et al., 1988) may allow to enlarge the antibody repertoire and to create even more resistant genotypes.

Search for marker genes and quantitative trait loci (QTL) affecting immune response and disease resistance

Other genes than MHC genes may be used to improve disease resistance. First candidates may be genes such as MHC which can be identified and are known to be associated with immune response and disease resistance. Within the MHC are already several genes from different classes whose respective function are not all yet clarified. Restriction fragment length polymorphism (RFLP) analysis of MHC may help in assigning effects to a more precise DNA fragment. Moreover, several polymorphic non-MHC genes, but closely linked to MHC genes, have already been described which might contribute to MHC disease associations (Guillemot, 1991). T-cell receptor genes may also be of interest knowing the importance of the cleft consisting in the MHC molecule, the T-cell receptor and the processed antigen in the immune response. Immunoglobulin allotype genes (Bacon et al., 1986), genes coding for surface alloantigen of B and T lymphocyte (Gilmour et al., 1986) and endogenous viral genes (Lamont et al., 1992) have been shown to be associated with disease resistance and immune response and thus could be also good candidates. Further on, more systematic research for mapping immune response and disease resistance genes should be performed. These QTL may be detected by building up saturated linkage maps. This consists of identifying evenly spaced markers containing the putative QTLs and assessing the relationship between the markers, and therefore the QTL, and a given trait, as well as the distance between the marker and the QTL. In the long run, brackets will become smaller by chromosome walking, and the QTL will be hopefully identified (Lander and Botstein, 1983; Soller, 1990). Finding polymorphic genetic markers may be achieved by using RFLP (see above), microsatellites and variable tandem repeat loci (VNTR) (Soller, 1990). Intensive research is now performed to determine the best strategy in terms of nature and size of data to reach the highest chance of finding QTLs. F2 and backcrosses of the H and L lines at selection limit could be an interesting material in this respect.

Specific or general disease resistance

Even if a certain immune parameter or a certain marker allele are indicators of resistance of a given disease, but are not valid for multiple pathogens, then the practical application of these relationships becomes limited. One major critics of the Biozzi experiment was that mice selected for high Ab response to SRBC were more resistant to extracellular pathogens but more susceptible to intracellular pathogens infection than low responders because of a reduced macrophage activity (Biozzi et al., 1984). Thus, in this case, selection for Ab production did not result in a general resistance to infections. Likewise chickens selected for high Ab production to SRBC were more resistant to MD, salmonellosis, coccidiosis and feather mites but more susceptible to E. coli and S. aureus infections than low responders (Gross et al., 1980; Dunnington et al., 1986). Another study showed also associations between resistance to some diseases but not all, still indicating that selecting simultaneously for resistance to several diseases might result in general resistance (Gavora and Spencer, 1983). In parallel, the risk of inducing resistance to specific disease by improving only one part of the immune system may be overcome by multitrait selection for different components of the immune system, like Ab response, macrophage activity, cell-mediated immunity. The progress is expected to be slower than in case of single trait selection but may confer a better overall immune system. At least two such multitrait selections have been initiated, in chickens (Cheng and Lamont, 1990) and in pigs (Mallard et al., 1990). Other authors claim that selection for early immune response would enhance a general resistance (Leitner et al., 1992). It will be very interesting to know the outcome of these experiments. In parallel, selecting for specific MHC alleles will be beneficial only if they are associated with resistance to several relevant diseases. Too few studies within one population (Bacon et al., 1981) allow to draw conclusions on this matter and comparison from different flocks (Bacon, 1987) are hampered by possible genotype by background interactions. Further on, some disadvantages may occur from reducing too much the number of alleles in a population if we consider the evolutionary theory that one reason for maintenance of polymorphism is the greater ability of animals to cope with a broad repertoire of pathogens, besides better fitness performances. As far as immune response is concerned, very high responders may have the disadvantage of always responding high regardless the conditions whereas

the low responders have a more adaptational capacity (Cohen et al., 1985). Therefore, the "immune profile" of an animal including its adaptational capacity should be considered rather than specific performances.

Antagonism with production traits

Finally, in a practical view, some attention and further research is needed to clarify the possible antagonism between genetic disease resistance (and favourable MHC alleles) and production traits. In cattle, negative correlations between disease and milk production have been found (Simianer et al., 1991). In chickens, associations and MHC effects are somewhat contradictory (Gavora et al., 1986; Bacon, 1987; Lamont et al., 1987; Kim et al., 1987; Abplanalp et al., 1992). Inconsistencies might result from a non direct effect or very small effect of MHC (or other immune response genes), but in linkage with QTLs affecting the production trait, so that the apparent effect of MHC depend on the linkage phase between MHC and the QTL. Still, several studies indicate a strong antagonism between body weight and resistance to MD (Gavora, 1990), and in the present selection lines, no phenotypic change occurred in level of reproduction or production traits, except for bodyweight which was higher in L line hens than in H line hens, the C line hens having intermediate weights. Multivariate analysis for Ab response and bodyweight of selection data or F2 individuals which were also MHC typed could clarify this possible genetic relationship. Antagonism between production and immune traits may be explained at the physiological level by a competition in the allocation of resources for the two traits (Martin et al., 1990). It is not reasonable to consider selecting for enhanced disease resistance only if negative correlated effects on economically important traits are expected. But on the other hand, ignoring disease traits in breeding programs may lead to increased susceptibility of animals to pathogens. Therefore, disease information should be included in breeding goals as it has been proven that improving simultaneously negatively correlated traits is feasible (Falconer, 1989). But further research is also needed to assess whether immune response genes are truly affecting production traits directly or not.

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SUMMARY

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INTRODUCTION

For economical and ethical reasons, improving genetic disease resistance may be an attractive preventive measure against infectious diseases in livestock production (General introduction). Genetic resistance to disease may be enhanced by direct selection, or by indirect selection using a correlated trait or identifiable genes associated with the disease trait. The aims of this study were

(1) to analyze in chickens the genetic control of (1a) **immune response**, specifically the antibody (Ab) response to sheep red blood cells (SRBC), and of (1b) **disease resistance**, specifically Marek's disease (MD), both with a special interest in the effects of **major histocompatibility complex** (MHC) genes,

(2) and to establish whether resistance to MD may be altered by selection for Ab response to SRBC.

GENETIC CONTROL OF AB RESPONSE TO SRBC

Divergent selection for Ab response to SRBC

Chickens from an ISA Warren cross were successfully selected for high (H) or low (L) primary Ab response to SRBC for 10 generations. In addition, a randombred control (C) line was kept. In chapter 1, primary and secondary titers were analyzed showing significant differences between the lines and higher titers in females than in males. Heritability of the Ab titer was estimated using an animal model by restricted maximum likelihood. The heritability estimate using data and relationships on all lines was .31. Major variation between years as shown by the C line was taken into account in estimating realized heritability of the Ab titer, producing estimates of .21 and .25 in the H and L lines, respectively. Therefore, these results confirmed the feasibility of altering Ab

response by selection and the polygenic control of Ab response. However, some specific genes may play a major role in the regulation of immune response. MHC genes were prime candidates in this investigation.

Identification and effects of MHC on Ab response to SRBC

Serology and biochemistry were used to identify MHC (B-) antigens in the H, C and L lines (Chapter 2). Moreover, newly identified types were compared with reference B-types. Serological typing was performed using antisera obtained by erythrocyte alloimmunization within the lines. Four serotypes were identified, called preliminarily B^{114}, B^{119}, B^{121} and B^{124} and were characterized for their B-G and B-F antigens by using SDS-PAGE and one-dimensional IEF respectively. This analysis showed similarities between some of the B-G or B-F types and reference types, but none of the haplotypes in the lines was identical for both B-G and B-F with the tested reference B haplotypes. Moreover, no recombinant between B-G and B-F was identified. Therefore, serology could differentiate haplotypes and be used for large scale typing in the following experiments.

Association between MHC and Ab response to SRBC was proven two ways. By MHC typing all animals (n=1602) from the ninth and tenth generations, it appeared clearly that selection for H and L Ab response had led to opposite changes in MHC type distribution in the H and L lines (Chapter 3). Whereas all types were present in the C lines, the B^{121} haplotype was predominant in the H line and the B^{114} haplotype was most frequent in the L line. To prove that these frequency changes resulted from MHC effects on the selected trait and not from drift or chance, MHC effects were estimated with an animal model in the H, C and L lines using data from the ninth and tenth generations and the relationships of all generations (Chapter 3). The effects were found significant and could be related partly to the MHC type frequency changes, but differed between lines. Moreover, these estimates could be biased because selection data from previous generations were not known. To overcome this problem, a F2 (n=1033) was produced from the H and L lines, displaying all MHC genotypes in equal number against a random background (Chapter 4). Estimates of MHC effects in the F2 were in agreement with the estimates in the selected lines and brought the final proof of a direct or closely linked effect of MHC on Ab response to SRBC.

EFFECT OF SELECTION FOR AB RESPONSE TO SRBC AND OF MHC ON RESISTANCE TO MD

From the ninth generations, an extra batch of H, C, and L female chicks (n=940), and F1 (HxL) female chicks (n=631) were produced and challenged for resistance to a virulent MD strain (Chapter 5). Heritability of mortality from MD was estimated by applying a threshold 'animal model-like'. And effects of MHC on mortality from MD were analyzed with a genetic epidemiological approach, using specifically a logistic regression analysis. Divergent selection for Ab response to SRBC led to a symmetric correlated response for time of death, but only to a downwards correlated response for resistance to MD: Chicks from the L and H line died respectively earlier and later than the C line chicks. And mortality in the L line was significantly higher than in the C line but mortality in the H line was not significantly lower than in the C line or than in the F1. Two reasons for this asymmetric response may be hypothesized: (i) genes associated with higher resistance to MD were in higher frequency in the base population, creating a higher genetic potential for selecting downwards, (ii) or genes having a negative effect on both antibody response to SRBC and resistance to MD are more numerous than those having a positive effect on both traits. Assumption (i) could be strengthened by a higher heritability of mortality to MD in the L line (.78) than in the H line (.45). If the assumption (ii) could be proved, selection for Ab response to improve genetic disease resistance will not be applicable. Attempts to estimate correlations between estimated breeding values for Ab response to SRBC and resistance to MD showed variation in estimates between lines, sexes, and whether MHC effect was taken into account or not. In parallel, resistance to MD could be modulated by MHC whose effect was moderate in the H line and most significant in the C line. But no clear relationship between effects of MHC on Ab response to SRBC (Chapter 3) and on mortality to MD (Chapter 5) could be established.

CONCLUSION

Both the Ab response to SRBC and resistance to MD showed in chickens a genetic potential for selection and high heritability values. Moreover, both traits were influenced by MHC, so that including MHC information may improve the efficiency of selection by applying marker assisted selection, or by selecting or introducing directly favourable alleles (General Discussion). On the contrary, this study could not prove that selection for Ab response to SRBC may be an effective indirect way of improving resistance to MD.

SAMENVATTING

SAMENVATTING

INLEIDING

Een verbeterde genetische resistentie tegen ziekten zou, om zowel economische als ethische redenen, een aantrekkelijke preventieve maatregel kunnen zijn tegen infectieuze ziekten in de veehouderij (General Introduction). Genetische resistentie tegen ziekte kan mogelijk verbeterd worden door directe selectie of door een indirecte selectie. Bij dit laatste kan gebruik worden gemaakt van gecorreleerde kenmerken of van identificeerbare genen die geassocieerd zijn met de ziektekenmerken.

De doelstellingen van de huidige studie waren
(1) het analyseren van de genetische regulering van (1a) de **immuunrespons**, specifiek ten aanzien van de produktie van antilichamen tegen schapen-rode-bloedcellen (SRBC), en van (1b) de **ziekte resistentie**, specifiek tegen de ziekte van Marek, beide met speciale aandacht voor de effecten van **major histocompatibiliteitscomplex** (MHC) genen, en
(2) vast te stellen of resistentie tegen de ziekte van Marek beïnvloed wordt door de selectie ten aanzien van de antilichaam respons tegen SRBC.

GENETISCHE REGULERING VAN DE ANTILICHAAM RESPONS TEGEN SRBC

Divergerende selectie ten aanzien van de antilichaam respons tegen SRBC

Kippen van een ISA Warren kruising werden succesvol geselecteerd op basis van een hoge (H) of lage (L) primaire antilichaam respons tegen SRBC gedurende 10 generaties. Bovendien werd een random gekruiste controle (C) lijn in stand gehouden. In hoofdstuk 1 worden de primaire en secundaire titers geanalyseerd. De lijnen tonen significante verschillen in antilichaam respons en de titers zijn hoger in de hennen dan in de hanen. De erfelijkheidsgraad van de antilichaam titer werd geschat met een diermodel

volgens de 'restricted maximum likelihood' methode. Wanneer gebruik werd gemaakt van de data en relaties van alle lijnen, bedroeg de schatting van de erfelijkheidsgraad 0.31. Correctie voor de aanzienlijke tussen-jaar variatie in de schatting van de gerealiseerde erfelijkheidsgraad van de antilichaam titer, zoals bleek in de C-lijn, leidde tot schattingen van 0.21 en 0.25 in respectievelijk de H en L lijnen. Deze resultaten bevestigden de mogelijkheid om de antilichaam respons te veranderen door middel van selectie en bevestigden bovendien de polygene regulatie van de antilichaam respons. Echter, mogelijk spelen een aantal specifieke genen een belangrijke rol in de regulatie van de immuun respons. De MHC genen leken hiervoor de belangrijkste kandidaat in de volgende experimenten.

Identificatie van MHC en effecten van MHC op de antilichaam respons tegen SRBC

Zowel serologische als biochemische technieken werden gebruikt om MHC-antigenen (B-complex antigenen) te identificeren in de H, C en L lijnen (Hoofdstuk 2). Bovendien werden nieuwe geïdentificeerde typen vergeleken met referentie B-typen. Bij de serologische typering werd gebruik gemaakt van antisera die verkregen werden door allo-immunisatie met rode bloedcellen binnen lijnen. Vier serotypen werden geïdentificeerd, die voorlopig B^{114}, B^{119}, B^{121} en B^{124} werden genoemd. Ten aanzien van hun B-G en B-F antigenen werden deze typen gekarakteriseerd met behulp van respectievelijk SDS-PAGE en ééndimensionale IEF. Uit deze analyses bleek dat er overeenkomsten tussen een aantal B-G of B-F typen en referentie typen waren, maar geen van de haplotypes in de lijnen was identiek voor zowel B-G als B-F met de geteste referentie B-haplotypes. Bovendien kon geen recombinant tussen B-G en B-F worden aangetoond. Er werd geconcludeerd dat op basis van serologische typering onderscheid gemaakt kan worden tussen haplotypes en dat de serologie gebruikt kan worden voor de grootschalige typering zoals die voor de overige experimenten nodig zou zijn.

Associatie tussen het MHC en de antilichaam respons tegen SRBC werd op twee manieren bewezen. Alle dieren (n = 1602) van de negende en de tiende generatie werden MHC getypeerd. Hieruit bleek duidelijk dat selectie op H en L antilichaam respons had geleid tot tegenovergestelde veranderingen in de frequentieverdeling van de MHC typen in de H en L lijnen (Hoofdstuk 3). Terwijl alle typen aanwezig waren in de C lijn, was het

B^{121} haplotype voornamelijk aanwezig in de H lijn en kwam het B^{114} haplotype het meest frequent voor in de L lijn. Om te bewijzen dat deze veranderingen in frequentie een gevolg waren van MHC effecten ten aanzien van het geselecteerde kenmerk en niet van 'drift' of kans, werden de MHC effecten geschat in de H, C en L lijn met een diermodel. Hierbij werd gebruik gemaakt van de data van de negende en de tiende generatie en de relaties tussen alle generaties (Hoofdstuk 3). De MHC effecten op de antilichaam respons bleken significant en konden deels gerelateerd worden aan veranderingen in de frequentieverdeling van MHC typen. Echter, deze effecten bleken te variëren tussen lijnen. Bovendien kunnen deze schattingen 'biased' zijn door het ontbreken van selectiegegevens van vorige generaties. Om dit probleem te omzeilen werd een F2-generatie (n = 1033) geproduceerd van de H en L lijnen, waardoor alle MHC genotypes,in gelijke aantallen tegen een random achtergrond konden worden getoetst (Hoofdstuk 4). Schattingen van de MHC effecten in de F2 kwamen overeen met de schattingen in de geselecteerde lijnen en vormden het uiteindelijke bewijs van een direct effect of een nauw gerelateerd effect van het MHC op de antilichaam respons tegen SRBC. In het F2 experiment werd 2,5 % van de phenotypische variatie verklaard door MHC genotypen.

EFFECT VAN SELECTIE VOOR ANTILICHAAM RESPONS TEGEN SRBC EN VAN MHC OP RESISTENTIE TEGEN DE ZIEKTE VAN MAREK

Van de negende generatie H, C en L lijnen werden 940 hennen geproduceerd. Van een kruising van de H en L lijnen werden 631 F1 hennen geproduceerd. Deze hennen werden onderworpen aan een challenge om de resistentie tegen een virulente stam van het Marek virus te meten (Hoofdstuk 5). De erfelijksheids graad op sterfte aan de ziekte van Marek werd geschat met een drempel "diermodel".De effecten van het MHC. op sterfte aan de ziekte van Marek werden geanalyseerd met een genetische epidemiologische benadering, hierbij werd met name een logistische regressie analyse gebruikt. Divergerende selectie op antilichaam respons tegen SRBC leidde tot een symmetrische gecorreleerde respons ten aanzien van het tijdstip van sterfte, maar alleen tot een negatieve gecorreleerde respons ten aanzien van de resistentie tegen de ziekte van Marek: hennen van de L en H lijn stierven respectievelijk eerder en later dan de hennen van de

C lijn. Bovendien was de sterfte in de L lijn significant hoger dan in de C lijn maar de sterfte in de H lijn was niet significant lager dan in de C lijn of in de F1. Voor deze asymmetrische respons werden twee hypothetische redenen aangevoerd: (1) genen die geassocieerd zijn met een hogere resistentie tegen de ziekte van Marek kwamen in een hogere frequentie voor in de basis populatie, waardoor een hogere genetische potentie ontstond voor een selectie in de L lijn, of (2) er zijn meer genen die een negatief effect hebben zowel op de antilichaam respons tegen SRBC als op de resistentie tegen de ziekte van Marek dan genen die een positief effect hebben op beide kenmerken. De juistheid van de eerste veronderstelling lijkt bevestigd te worden door de hogere erfelijkheidsgraad van sterfte tegen de ziekte van Marek in de L lijn (0.78) dan in de H lijn (0.45). Als de tweede veronderstelling bewezen kon worden, zou dit betekenen dat er geen mogelijkheid bestaat om selectie op antilichaam respons toe te passen om een verhoogde genetische resistentie tegen ziekten te krijgen. Pogingen om correlaties te schatten tussen geschatte fokwaardes ten aanzien van antilichaam respons tegen SRBC en resistentie tegen de ziekte van Marek liet zien dat er variatie bestond in de schattingen tussen lijnen, sexes en MHC. Parallel hieraan bleek dat de resistentie tegen de ziekte van Marek werd gemoduleerd door het MHC. Het effect was gematigd in de H lijn en was sterk significant in de C lijn. In de C-lijn werd 3 % van de phenotypische variatie verklaard door MHC genotypen. In dit experiment kon echter geen sterke relatie worden aangetoond tussen effecten van het MHC op enerzijds de antilichaam respons tegen SRBC (Hoofdstuk 3) en anderzijds de sterfte als gevolg van de ziekte van Marek (Hoofdstuk 5).

CONCLUSIE

In kippen bleek zowel de antilichaam respons tegen SRBC als de resistentie tegen de ziekte van Marek een genetisch handvat te zijn om te selecteren. Beide hadden een hoge erfelijkheidsgraad. Bovendien werden beide kenmerken beïnvloed door het MHC. Daarom zou het opnemen van MHC informatie de efficiëntie van selectie kunnen vergroten. Dit door de toepassing van marker geassocieerde selectie of door selectie of introductie van direct positieve allelen (General Discussion). In deze studie kon niet bewezen worden dat selectie voor antilichaam respons tegen SRBC een indirecte effectieve methode is om de resistentie tegen de ziekte van Marek te verbeteren.

RESUME

RESUME

INTRODUCTION

Pour des raisons économiques et éthiques, il peut être intéressant d'améliorer génétiquement la résistance aux maladies des animaux en tant que mesure préventive contre les maladies infectieuses (Introduction Générale). La résistance aux maladies peut être améliorée par sélection directe sur le caractère, ou bien par sélection indirecte en utilisant un caractère corrélé ou des gènes identifiables et associés avec le caractère de résistance. Les objectifs de cette étude étaient

(1) d'analyser chez la Poule le contrôle génétique de:

(1a) la **réponse immunitaire**, et en particulier la réponse en anticorps à des globules rouges de mouton (GRM),

(1b) la **résistance aux maladies**, et en particulier la résistance à la maladie de Marek, avec dans les deux cas un intérêt particulier pour les effets des gènes du **complexe majeur d'histocompatibilité** (CMH),

(2) et d'établir s'il serait possible de modifier la résistance à la maladie de Marek par sélection sur la réponse en anticorps aux GRM.

CONTROLE GENETIQUE DE LA REPONSE EN ANTICORPS AUX GRM

Sélection divergente sur la réponse en anticorps aux GRM

Des poules issues d'un croisement ISA Warren ont été sélectionnées avec succès sur la réponse primaire en anticorps haute (H) ou basse (B) à des GRM pendant 10 générations. De plus, une lignée témoin (T) a été maintenue. Dans le Chapitre 1, les titres primaires et secondaires en anticorps ont été analysés, montrant des différences significatives entre les lignées ainsi que des titres plus élevés chez les femelles que chez les mâles. L'héritabilité de le réponse en anticorps aux GRM a été estimée en utilisant

un modèle animal et la méthode du maximum de vraisemblance restreint. L'héritabilité ainsi estimée en utilisant toutes les données et les relations de parenté des trois lignées était de 0,31. Des fluctuations majeures de la réponse en anticorps suivant les années, comme l'a montré la lignée T, ont été prises en compte dans l'estimation de l'héritabilité réalisée de la réponse en anticorps, donnant des valeurs estimées de 0,21 dans la lignée H et 0,25 dans la lignée B. Par conséquent, ces résultats ont confirmé la possibilité de modifier le niveau de la réponse immunitaire par sélection, ainsi que le caractère polygénique de la réponse en anticorps. Cependant, il était possible que certains gènes jouent un rôle particulier dans la régulation de la réponse immunitaire. Dans cette investigation, les gènes du CMH étaient, à ce titre, des candidats de choix.

Identification et effets des gènes du CMH sur la réponse en anticorps aux GRM

Les techniques sérologiques et biochimiques ont été utilisées afin d'identifier les antigènes du CMH (appelés aussi antigènes du complexe B) dans les lignées H, T et B (Chapitre 2). De plus, ces nouveaux types B ainsi identifiés ont pu être comparés avec les types B de référence. Le typage sérologique a été effectué en utilisant des antisérums obtenus par alloimmunisation avec des globules rouges intra-lignée. Quatre sérotypes ont été ainsi identifiés et appelés provisoirement B^{114}, B^{119}, B^{121} et B^{124}. Ces sérotypes ont été ensuite caractérisés pour leurs antigènes B-G en utilisant la technique SDS-PAGE et pour leurs antigènes B-F par IEF. Cette analyse a révélé des similitudes entre certains des types B-G ou B-F présents dans les lignés et les types de référence; cependant, aucun des haplotypes présents dans les lignées n'était identique, à la fois pour B-G et B-F, avec les types de référence B testés. De plus, aucune recombinaison entre les régions B-G et B-F n'a pu être identifiée. Par conséquent, la sérologie développée ici était en mesure de différencier des haplotypes du CMH uniques pour les différentes régions, et pouvait ainsi être utilisée pour le typage CMH à grande échelle dans les expériences suivantes.

L'association entre le CMH et la réponse en anticorps aux GRM a été démontrée dans les deux sens. Le typage CMH de tous les animaux (n=1602) des générations 9 et 10 a montré clairement que la sélection sur la réponse immunitaire H et B avait conduit à des distributions opposées des types du CMH dans les lignées H et B (Chapitre 3). En effet, alors que tous les types étaient présents dans la lignée T, l'haplotype B^{121} était

prédominant dans la lignée H et l'haplotype B^{114} était le plus fréquent dans la lignée B. Afin de prouver que ces changements de distribution était dûs aux effets du CMH sur le caractère sélectionné, et non pas à la dérive génétique ou le hasard, les effets du CMH ont été estimés en retour sur la réponse en anticorps aux GRM dans les lignées H, T et B en utilisant un modèle animal; dans cette analyse, les données des générations 9 et 10, ainsi que les relations de parenté de toutes les générations ont été utilisées (Chapitre 3). Les effets du CMH sur la réponse immunitaire ainsi estimés se sont révélés significatifs, et pouvaient expliquer en partie les changements de fréquence des types du CMH pendant la sélection. Mais ces effets étaient variables suivant les lignées. De plus, ces estimations des effets du CMH pouvaient être biaisés dans la mesure où les données concernant les générations précédentes n'avaient pu être utilisées puisque les types du CMH n'étaient alors pas connus. Afin de résoudre ce problème, une F2 (n=1033) a été produite à partir des lignées H et B; dans la F2, tous les génotypes du CMH étaient représentés en nombre égaux et dans un contexte aléatoire (Chapitre 4). Les estimations des effets du CMH sur la réponse immunitaire dans la F2 étaient en accord avec les estimations obtenues précédemment dans les lignées sélectionnées, et apportaient ainsi une preuve définitive d'un effet direct ou indirect (dûs à un lien génétique) des gènes du CMH sur la réponse en anticorps aux GRM. Dans la F2, le génotype du CMH expliquait 2,5% de la variance phénotypique.

EFFETS DE LA SELECTION SUR LA REPONSE EN ANTICORPS AUX GRM ET DU CMH SUR LA RESISTANCE A LA MALADIE DE MAREK

Issues des lignées H, T et B de la génération 9, 940 femelles ont été produites, et à partir d'un croisement F1 des lignée H et B de la génération 9, 631 femelles ont été obtenues; ces femelles ont été soumises à un test de résistance à la maladie de Marek par contact avec une souche active de virus (Chapitre 5). L'héritabilité de la mortalité par la maladie de Marek a été estimée en utilisant un "modèle animal" à seuil. Et les effets du CMH sur la mortalité par la maladie de Marek ont été estimés à l'aide d'une approche génétique et épidémiologique, en utilisant une analyse de régression logistique.

La sélection divergente pour la réponse en anticorps aux GRM s'est accompagnée

d'une réponse symétrique corrélée en ce qui concerne l'âge à la mort dans les lignées H et B, mais d'une réponse corrélée en ce qui concerne la mortalité totale dans la lignée B seulement. En effet, les animaux de la lignée B sont morts plus tôt et ceux de la lignée H sont morts plus tard que les animaux de la lignée T. Et la mortalité était significativement plus élevée dans la lignée B que dans la lignée T, alors que la lignée H n'était pas significativement plus résistante que la lignée T. La F1 était aussi résistante à la maladie de Marek que la lignée H.

Deux raisons pour cette asymétrie peuvent être avancées: (i) les gènes associés à une résistance accrue à la maladie de Marek étaient plus nombreux dans la population de base, créant ainsi un potentiel génétique plus élevé dans le sens d'un accroissement de la susceptibilité, (ii) ou bien les gènes ayant un effet négatif à la fois sur la réponse en anticorps aux GRM et sur la résistance à la maladie de Marek étaient plus nombreux que ceux ayant un effect positif sur les deux caractères. L'hypothèse (i) pouvait être renforcée par le fait que l'héritabilité de la mortalité par la maladie de Marek était plus élevée dans la lignée B (0,78) que dans la lignée H (0,45). Si l'hypothèse (ii) s'avérait être exacte, la possibilité de sélectionner sur la réponse en anticorps en vue d'améliorer la résistance génétique aux maladies ne serait pas applicable. Les corrélations entre les valeurs génétiques pour la réponse en anticorps aux GRM et pour la résistance à la maladie de Marek ont été estimées et variaient suivant la lignée, le sexe, et suivant que les valeurs génétiques étaient corrigées pour le génotype du CMH ou non.

Parallèlement, la résistance à la maladie de Marek était influencée par le CMH, dont l'effet était modéré dans la lignée H et le plus significatif dans la lignée T. Dans la lignée T, le génotype du CMH expliquait 3% de la variance phénotypique. Mais aucune relation entre les effets du CMH sur la réponse en anticorps aux GRM (Chapitre 3) et ceux sur la mortalité par la maladie de Marek (Chapitre 5) n'a pu être clairement établie à partir de ces expériences.

CONCLUSION

La réponse en anticorps aux GRM ainsi que la résistance à la maladie de Marek ont montré des potentiels génétiques pour la sélection et des valeurs élevées d'héritabilité. De plus, ces deux caractères sont influencés par le CMH, si bien que l'utilisation du type du CMH dans la sélection pourrait accroitre l'efficacité de la sélection, soit en appliquant une sélection combinée sur le caractère et le ou les marqueurs associés, soit en sélectionnant ou en introduisant directement des allèles favorables (Discussion Générale). Par contre, cette étude n'a pas pu prouver que la sélection sur la réponse en anticorps aux GRM puisse être une manière efficace d'améliorer la résistance à la maladie de Marek.

Curriculum Vitae

Marie-Hélène Pinard was born June 13, 1965 in Rueil Malmaison, France. In 1982, she received the School leaving certificate ('Baccalaureat') majoring in Mathematics ('serie C'). After the preparatory courses ('classes préparatoires d'entrée aux grandes écoles') (1982-1985), she entered the National Engineer Agronomic High School ('Ecole Nationale Supérieure Agronomique') of Montpellier where she studied two years (1985-1987). She specialized the third year at the Agronomic National Institut ('Institut National Agronomique') of Paris-Grignon in "Sciences and technics of Animal Production" and "Genetic Improvement" and received in September 1988 her degree of Agronomic Engineer (Ingénieur Agronome). Admitted at the National Institut of Agronomic Research ('Institut National de la Recherche Agronomique'), she entered the laboratory of factorial genetics (Laboratoire de Génétique Factorielle) in Jouy-en-Josas in December 1988. In March 1989, she was sent to perform her PhD at the Agricultural University of Wageningen in the Department of Animal Husbandry. During three years, she conducted a research on selection for immune response in chickens, resulting in the present work. Since July 1992, she is back at the INRA Laboratory of Factorial Genetics in Jouy-en-Josas (France).