QTL MAPPING EXPERIMENT IN F2 CROSS OF CHICKENS DIVERGENTLY SELECTED FOR ANTIBODY RESPONSE TO SRBC

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INTRODUCTION

Infectious diseases are responsible for major economic losses in livestock production. Although control of the environment by sanitation and isolation, and massive use of vaccination and drugs, reduces the incidence of many diseases, the problem has not been eliminated (Heller *et al*., 1992). Genetic resistance may be improved indirectly by selecting for a broad immune response. Chickens selected for high antibody responses to sheep red blood cells (SRBC) showed higher resistance to some infectious diseases e.g. Marek's and Newcastle diseases (Gross *et al*., 1980 ; Dunnington *et al*., 1986). However, so far no selection experiment has resulted, in enhanced general resistance or enhanced immunity (Heller *et al*., 1992). Little is known of the genetic basis that underlay the innate and acquired disease resistance. Selection and crossing are procedures that may be used in genetic analysis of quantitatively inherited traits in poultry. The use of the first (F1) generation and second (F2) generation crosses allow for a check on the presence of heterosis (Miller *et al*., 1992). The use of molecular genetic markers can be of great help to identify genomic regions encoding immune response genes. The aim of this paper is to present a first data of QTL mapping experiment and estimated heritabilities of different immune parameters.

MATERIAL AND METHODS

Experimental design, creation of the F2 population. The F2 population originates from a cross (ISA Warren) between two divergently selected lines for either high (H line) or low (L line) primary antibody response to sheep red blood cells (SRBC) at 5 days after primary intramuscular immunisation with SRBC at 37 days of age (Van der Zijpp and Nieuwland, 1986). From the 17th generation, 15 males from the L line and 15 females from the H line and 16 males from the H line and 16 females from the L line were selected to produce reciprocal crosses. From the F1 generation, 12 males and 24 females were chosen from HxL and LxH crosses to produce 1240 F2 animals, with an average of 52 offspring per full - sib family. Hens and cocks were housed in rearing cages with free access to feed and water.

Phenotyping of the F2 population. The phenotypic data for the immunological responses have been determined. Blood samples were collected from birds at the day 0 and the test day for relevant antigens i.e. primary antibody response against SRBC at 35 days, *E.coli* at 55 days, KLH-DNP (keyhole limpet haemocyanin – dinitrophenyl) at 82 days , *Mycobacterium butyricum* at 103 days, secondary antibody response against SRBC at 127 days and cellular response against ConA (ConcanavalinA) at 132 days of age. The Ab response to SRBC was determinated by agglutination as a $\log_2$ of the reciprocal of the highest serum dilution giving

complete agglutination. Total Ab titres to *E.coli*, KLH–DNP (Cal Biochem – Novabiochem) and *M.butyricum* (Difco Lab) were determinated by ELISA. Titres were expressed as the $\log_2$ values of the highest dilution giving a positive reaction. Cellular response against ConA (Sigma) was determined according to method described by Sijben *et al.* (2000) and expressed as a SI (stimulation index) = mean counts per minute in stimulate cultures/mean counts per minute in unstimulate cultures.

Genotyping of the F2 population. In total 174 microsatellite markers were chosen, distributed over the chicken genome, approximately 20 centiMorgan (cM) apart. PCR reactions were performed in a total volume of 12 µl containing 10 to 60 ng genomic DNA, 25 mM $MgCl_2$, 50 mM KCl, 10mM Tris-HCl pH = 8.3, 1 mM tetramethylammonium chloride (TMAC), 0.1 % Triton X-100, 0.01 % gelatin, 200 µM dNTP, 0.25 U Goldstar polymerase (Eurogentech), 2.3 pmole of each primer and covered with 10 µl mineral oil (Sigma). PCR programme used was : 2 min at 95°C, 35 cycles of 30 s at 95°C, 30 s (annealing temperature 45°C - 60°C), and at 72°C, and followed by an elongation step of 3 min 30 s 72°C. PCR products were pooled in a total volume of 50 µl. A mixture of 1 µl pooled PCR product with 1.6 µl loadingbuffer (containing 80 % formamide and GENESCAN – 350 TAMRA) was loaded on 6 % denaturing polyacrylamide gel (Sequagel 6, National Diagnostics) on an ABI 373. Fragment sizes were analysed with GENESCAN fragment analysis software (Perkin – Elmer, Applied Biosystems). Allele calling was performed using GENOTYPER 2.0 software (Perkin – Elmer, Aplied Biosystems). In total 722 animals have been genotyped.

Statistical analysis. SAS software package was used to investigate the basic statistics of the traits (SAS, 1982). Heritabilities were estimated using ASREML (Gilmour *et al*., 2000) according to the following model :

$$Y_{ijk} = \mu + SEX_i + BATCH_j + A_k + e_{ijk}$$

Y_{ijk} – the individual observation of variable ;
μ - the average of the trait observed ;
SEX_i – the fixed effect for the i' sex (i = 1,2) ;
$BATCH_j$ – the fixed effect for the j'batch (j = 1,2,...6) ;
A_k – the random effect of the k'th animal (k = 1,2.....1343) ;
e_{ijk} – the residual effect

RESULTS AND DISCUSSION

Means and standard deviations (SD) of titres are presented in table 1.

In our F2 population the average antibody titre to the SRBC primary response was 6.80 ± 2.94, Pinard and van der Zijpp (1993) found 3.77 ± 1.13 measured on 1000 animals. The remaining traits have only been measured in present F2 generation.

It has been suggested by Boa–Amponsem *et al*. (1997) that there is a sex- linked effect on the Z chromosome, for female progeny, for antibody titres against SRBC. Therefore we investigated if there was a difference between HxL and LxH groups for the different immunological traits. In table 2 we present means and SD for hens in reciprocal crosses.

Table 1. Mean and SD of antibody responses to *M.butyricum*, KLH, *E.coli*, SRBC (primary and secondary) and cellular response against ConA

Antigen	N	Mean±SD
MycoB	1068	4.87 ± 1.36
KLH	1192	5.05 ± 2.08
E.coli	1165	6.03 ± 1.77
SRBC primary	1172	6.80 ± 2.94
SRBC secondary	1154	4.94 ± 1.61
ConA	959	10782.73 ± 7967.56

Table 2. Mean and SD of the antibody response to *M.butyricum*, KLH, *E.coli*, SRBC (primary and secondary) and cellular against ConA for F2 hens in reciprocal crosses

Antigen	Mean±SD	
	HxL	LxH
MycoB	4.80 ± 1.32	5.24 ± 1.21*
KLH	5.01 ± 2.11	5.34 ± 2.20
E.coli	6.11 ± 1.59	5.98 ± 1.80
SRBC primary	7.33 ± 2.85	7.00 ± 3.06
SRBC secondary	5.29 ± 1.39	5.17 ± 1.52
ConA	9682.22 ± 7808.93	14366.89 ± 8439.00*

*Indicates significant difference ($P < 0.05$) between reciprocal crosses

In the experiment of Boa-Amponsem *et al*. (1997) significant sex linked effect has been observed for the antibody response against SRBC. In our study only antibody response for *M.butyricum* and cellular response for *ConA* were significantly affected by the sex chromosome.

We observed high variability for antibody responses for different antigens inside the full-sib families and in between them. Figure 1 shows an example of antibody titre distribution to SRBC.

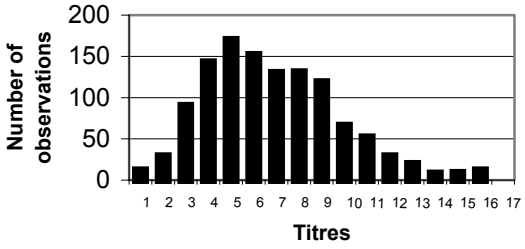

Figure 1. Distribution of primary antibody titres against SRBC in F2 (N = 1172)

The distribution is almost normal with the mean around 6, but nevertheless high (16) and low (1) antibody titres within the progeny were found.

The phenotypic variance and heritability were investigated for immune antigens in F2 (table 3). The highest heritability is for ConA and the lowest for KLH. For both primary and secondary SRBC responses, h^2 were similar.

Table 3. Phenotypic variance and heritability of the antibody response to *M.butyricum*, KLH, *E.coli*, SRBC (primary and secondary), and cellular response against ConA

Antigen	σ^2_p	$h^2 \pm SE$
MycoB	1.50	0.17 ± 0.06
KLH	2.64	0.07 ± 0.03
E.coli	2.40	0.24 ± 0.08
SRBC primary	5.27	0.14 ± 0.05
SRBC secondary	2.39	0.14 ± 0.06
ConA	$0.57 * 10^8$	0.40 ± 0.11

QTLs identification require two conditions : resource population has to derived from parental lines genetically different for the trait of interest ; and linkage disequilibrium. In our experimental design both conditions are present, so we can conclude that by using the genotypic and phenotypic data we should be able to identify genomic regions encoding immune response genes.

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