HETEROSIS, IMMUNE RESPONSE AND DISEASE IN POULTRY

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SUMMARY AND CONCLUSIONS

For a variety of infectious diseases and immuno-logical parameters heterosis has been shown. Because F2 generations and backcrosses are not often produced the relative importance of dominance versus epistasis cannot be decided. In poultry the Major Histocompatibility Complex (MHC) has been studied serologically, biochemically and molecularly in diverse background genomes. Interaction between MHC and background genome has frequently been observed for immune response, Marek disease and lymphoid leukosis. With the advent of genomic genetics not only relevant genes for immune response and disease can be easily detected, but also the interactions between genes at a much larger scale. Some examples of complementation, at cis- and trans-level are available for tumour regression. Germline transmission of genes may therefore provide a suitable model for study of gene interaction at molecular and gene product level.

Studies of heterosis and epistasis need cautious interpretation with regard to importance of maternal antibodies and environmental effects, like route of infection or vaccination. Genomic genetics again may be helpful to locate loci that have an advantage in defined environments.

In general we have new molecular tools available to recognise interaction between genes. Our challenge will be to understand the concomitant biochemical and physiological (immunological) mechanisms and relate these to disease.

HETEROSIS AND EPISTASIS

Crossing of lines, inbred or not, may lead to the phenomenon heterosis, measured as the difference between the crossbred and means of the (inbred) parent population. Falconer (1981) showed that the amount of heterosis can be expressed as $HF1 = \Sigma\, dy^2$. In this equation d represents the genotypic value and y the difference of gene frequency between the two populations. Two important conclusions from his presentation are that no heterosis may be observed, because it is dependent on directional dominance, and that the amount of heterosis is specific to each particular cross. Falconer (1981) then proceeds to utilize information from the F2 generation, the offspring of randomly mated F1 parents. The amount of heterosis here is $HF2 = \Sigma\, 1/2dy^2$, provided that epistatic interaction is absent.

Hill (1982) elucidated, that differences between midparent $\bar{P}$, F1 and F2 originate in differences in gene frequencies and gene effects. The F1 and $\bar{P}$ differ by dominance and additive interactions, but F1 and F2 differ by dominance and interactions with dominance. Studies of F2 populations should therefore consider the different possible sources of epistatic interactions besides dominance.

OVERDOMINANCE AND FITNESS

The potentialities for crossbreeding are greatest when there is much non-additive, i.e. dominance and epistasis, and little additive variance. Of special importance is dominance, especially overdominance, for fitness traits. Included in fitness is disease resistance (see Falconer, 1981). Considering the numerous disease agents it seems plausible that chickens have a system of defense, that has an optimal function when it is also extremely variable in function and structure. When immune functions are genetically determined, a significant contribution of heterozygote advantage might be expected. Because it is extremely difficult to dose disease the testing of this hypothesis has been very limited. Overdominance for fitness maintains an equilibrium gene frequency at intermediate levels. This is an attractive explanation for polymorphism as reflected in the Major Histocompatibility Complex (B-complex) of the chicken (Guillemot et al., 1989). Assuming that the genetic polymorphism of the B-complex is functionally related to the immune response, then the presence of the B-complex is most likely maintained by diversity in infectious diseases and their appearance over a chick's life time and generations.

Siegel and Gross (1980) reciprocally crossed lines selected for antibody production and for persistence of antibody titers. They compared the antibody titers of crossbred progeny with the next (third) selection generation of lines. The F1 crosses showed evidence for non-additive genetic variation for both selection traits. The mean antibody titers of the crosses was significantly greater than the mid-parent means. Reciprocal effects were observed for the persistency only.

Ubosi et al. (1985a) reported on reciprocal crosses of the 9th generation of the same lines. In these experiments age, dose of antigen, antibody titers and body and organ weights were included. Here the antibody titers of crosses were lower than the mid-parent average. At low dose levels of antigen the line selected for low antibody response responded less frequently. The authors discussed that dominance is indicated for these threshold responses. For relative bursa weights the crosses were higher than the mid-parent values. For relative spleen weight the crosses were not significantly different from the high line and thymus weight behaved like an intermediate.

Ubosi et al. (1985b) presented data of 9th generation crosses for primary and secondary inmmune response. Crosses were intermediate to those of the parental lines in the primary response; in the secondary response no significant difference between lines existed. There was a dependency on dosage which in the secondary response resulted in reciprocal effects at the lowest dose level. Mitogenic responses to phyto-haemagglutinin (PHA) and concanavalin A (ConA) have been used to determine cell-mediated immunity. Lossila et al. (1979) presented evidence for negative heterosis in the PHA response, but not for ConA. Unfortunately, there were no reciprocal crosses available, so no further inference is possible.

Briles et al. (1982) studied the incidence of Marek disease in five B genotypes, resulting from a cross of two purebred Leghorn lines. Five genotypes were possible, with the B2/B21 combination being the most resistant. The B21 allele was present in the female line, but not in the males of the other line. B21 homo-

zygotes usually show greatest resistance, but this study shows that the B2/B21 heterozygote may show synergism. In a study of Bedouin and White Leghorn breeds Heller et al. (1981) reported intermediate immune responses to vaccines for Newcastle disease, fowl-pox and E.coli in the reciprocal crosses. Rate of development of titer to E.coli was significantly more rapid in Bedouin chick compared to Leghorns and reciprocal crosses.

GENOMIC GENETICS

Briles et al. (1977) presented a classical study about the effect of B-complex alleles on resistance to Marek's disease. In these association or linkage studies backcrossing of F1 to parental genotypes (see Briles et al., 1977), recombinants (Pevzner et al., 1978; Briles et al., 1983), recombinants in F1 and F2 populations (Collins et al., 1985) are utilised. In the above studies the B-complex alleles are serologically defined, but also the sex-linked dwarfing gene has been studied (Klingensmith et al., 1983).

With the advent of identification of genetic polymorphisms at DNA level - restriction fragment length polymorphisms and oligonucleotide polymorphisms-marker based analysis in segregating populations has become possible at a large scale. Genomic genetics (Soller and Beckman, 1988) can be helpful to understand the genetics of the trait, mapping and characterisation of the alleles and in providing specific markers for future breeding programmes. Marker-based analyses in segregating populations is especially interesting for disease, where natural and artificial selection (survival breeding) can lead to resistant and susceptible populations. The difference between these populations is most likely due to difference in allelic frequencies and not in allelic composition. According to F2 mating type detection of marker - quantitative trait loci (QTL) linkage may be determined. The proportion of matings of each sort will depend on allele frequencies in the two populations. Also the number of F2 individuals will have to be large to be able to detect the loci with powerful effects on the trait. Hillel et al. (1989) have revealed a series of useful DNA fingerprints in poultry. The total number of DNA polymorphic loci can crease dramatically with this technique besides the already known immunological and biochemical polymorphisms. The B-complex, the MHC, can also be genotyped molecularly with specific DNA probes now (Chaussé et al., 1989).

A non-DNA, but serological example of the MHC:B-G haplotype-frequencies in chicken lines selected divergently for the humoral immune response is presented by Pinard et al. (1990) at this conference. Four B-haplotypes are still present in the lines, with B121 available only in the high and control line. B114 is most frequent in the low line and B119 and B124 at intermediate levels in all lines (Van der Zijpp et al., 1988). An F2 generation of these lines has been planned in order to study the quantitative effects of the MHC on immune response and Marek disesase mortality, provided sufficient numbers of each mating type can be produced. The result of this experiment may explain the divergence in B-haplotypes between the selected lines. However, Hartmann (1988) reviewed various examples of interaction between the B-complex and other genes in crosses for Marek disease mortality. Even when the B-complex has been detected as a major gene in our lines the

results of crossing with other lines need to be evaluated in future.

GERMLINE TRANSMISSION

Another type of 'crossbreeding' has been introduced with the techniques for gene transfer. Depending on the technique the DNA has become inserted in the germ line or a chimaera is produced, that may transfer the desired DNA to the next generation. The cloned DNA may come from the same breed, species or other species. In poultry there is great interest in the application of gene manipulation (Wentworth et al., 1989; Shoffner, 1986; Salter et al., 1986) for disease resistance. How the inserted gene interacts with the present genome is not known. Breeding evaluation for expression, stability, and testing for many traits are necessary to understand the presence of interactions, which could be comparable to heterosis and epistasis. An example of different effects in crossbred chicks for the MHC is given below (Table 1). It appears that apart from possibly maternal effects the interactions between MHC-genotypes and background genes affecting mortality to Marek disease can be quite important. These examples indicate negative heterosis. The question how the B-21 linked resistance gene relates to other genes remains unanswered. The effect of insertion of a B-21 linked resistance gene may be dependent on the present genome (with B-21 missing and therefore generally more susceptible to Marek disease).

Gebriel and Nordskog (1983) compared segregating B-complex alleles against different genetic backgrounds for resistance to Rous sarcoma virus. The results verified that cellular resistance is not linked to the B-complex, but tumour regression is associated with the MHC. The dual mechanism of resistance is important when transfection of resistance genes is comtemplated.

Congenic lines have been developed to study the effect of, usually, B-haplotypes against a common background. In experiments with 8 B-congenic lines, developed in East Lansing, Lillehoj et al. (1989) found that the immunity to Eimeria tenella is controlled by interaction between MHC-genes and non-MHC-linked genes. The outcome of host response to E.tenella infection is therefore not easily manipulated by B-alleles alone.

Martin et al. (1989) somewhat surprisingly found heterozygote advantage for resistance to Marek's disease, based on B13 and B21-haplotypes in their high and low antibody response lines. Their comparison of haplotypes between lines showed line (background genome) by genotype (MHC) interactions and also for sex by genotype. For the antibody response to sheep red blood cells the backcross of F1 to high and low antibody response lines had produced also interactions between B-haplotypes and background genome (Dunnington et al., 1989).

DIVERSITY OF RESPONSE AND ENVIRONMENT

Powell (1987) reviewed innate mechanisms and acquired immunity for resistance to infectious diseases. The resistance to infectious agents is complex; immuno competence is governed by many interactions between cells and humoral factors. Immuno competence is dependent on genotype and environment, where a lifelong inter-

action with antigens determines immune response. Stress also contributes to the response pattern (Van der Zijpp et al., 1989; Gross and Siegel, 1988)). For the evaluation of heterosis for immune response traits in the chicken genotype-environment interaction may be important. Although possibly not seen at the level of particular heterozygote combinations the overall better performance of hybrids could be explained by heterozygotes being more effective in raising an immune response to a large variety of diseases. This should result in less frequent disease problems in hybrid animals. This hypothesis is difficult to prove with experiments or with datasets from practice.

Hartmann and Sanz (1971) observed that crossbreds, in experiments involving 13.000 chicks, died at a slower rate after infection. This was even clearer when crossbreds were infected by contact and not by peritoneal injection. Effect of sex was more pronounced in crossbreds than in purebreds, but also dependent on type of infection. The effects of infection procedure on heterosis were that heterosis was clearly present after natural infection, but not after intraperitoneal infection.

Gavora and Spencer (1983) organised a very large experiment to test strains and crosses for general and specific combining ability in two environments: with and without vaccination. When chicks were not vaccinated crosses showed heterosis for Marek disease mortality. When chicks were vaccinated this heterosis virtually disappeared. It is interesting to note that for hen-housed egg production the vaccination by selection goal (i.e. egg production and resistance) interaction was highly significant.

MATERNAL ANTIBODIES

When evaluating disease and immune response in crossbred chicks, the results may be affected by maternal antibodies. Reciprocal crosses sometimes show large differences in disease frequency and/or mortality (Tables 1 and 2). The role of antibodies in resistance to specific diseases is often not known. Nordskog and Pevzner (1977) discussed mortality in general and related to specific diseases. They hypothesised that maternal antibodies and/or sex linkage are responsible for different results in reciprocal crosses.

Genetically resistant hens may not need to produce maternal antibodies, because other defense systems were effective early in the response. These maternal antibodies are, however, of vital importance to protect the young chicks for some diseases like lymphoid leukosis (Nordskog and Pevzner, 1977).

In a study of two resistant White Plymouth Rock (WPR) lines and a susceptible commercial WPR line the reciprocal crosses showed significant differences in mortality to Marek disease. Although the crosses were intermediate to that of both parent lines, the cross with resistant male parents was considerably more resistant. Hartmann (1989) reported on leukosis mortality in reciprocal crosses. Although the R-line is very resistant to both subgroups A and B and the G-line also to subgroup B and the M-line is quite susceptible to both viruses, crosses with R-female parents were more susceptible even than the susceptible M-line by itself. The results can therefore be understood by lack of protection by maternal antibodies to subgroup A of the R-line.

Table 1. Reciprocal crosses of B19 and B21 haplotypes and mortality caused by Marek disease in lines A and B.

Sire Genotype	Line	Dam Genotype	Line	Progeny Genotype	Mortality %
B21/21 (n=15)	A	B21/21 (n=25)	A	B21/21 (n=110)	16
	A	B19/19 (n=15)	A	B21/19 (n=36)	22
B19/19 (n=11)	A	B19/19 (n=45)	A	B19/19 (n=97)	42
	A	B21/21 (n=22)	A	B19/21 (n=43)	37
B21/21 (n=10)	B	B21/21 (n=13)	B	B21/21 (n=54)	9
	B	B19/19 (n=21)	B	B21/19 (n=50)	30
B19/19 (n=11)	B	B19/19 (n=14)	B	B19/19 (n=77)	60
	B	B21/21 (n=33)	B	B19/21 (n=93)	49

from Blankert et al., 1990

Table 2. Effects of B19 and B21 haplotypes in different genomes.

Sire line C (n=30) Genotype	Dam (n=60) Genotype	Line	Progeny Genotype	Mortality %
B5/19	B19/19	A	B5/19-B19/19 (n=230)	58
B5/19	B21/21	A	B5/21-B19/21 (n=182)	46
B5/19	B19/19	B	B5/19-B19/19 (n=130)	77
B5/19	B21/21	B	B5/21-B19/21 (n=159)	61
B15/15	B19/19	A	B15/19 (n=194)	53
B15/15	B21/21	A	B15/21 (n=191)	51
B15/15	B19/19	B	B15/19 (n=162)	73
B15/15	B21/21	B	B15/21 (n=186)	68

from Blankert et al., 1990

COMPLEMENTATION

Hill (1982) discussed recombination loss in terms of interaction solely between pairs of genes, both at the same loci and at different loci. Here interactions between cis-acting genes are ignored.

Jones et al. (1978) observed that proteins of the mouse class II MHC, dimers with α and β chain, showed complementation in both cis- and trans-situation. When α and β subunits from different haplotypes mix, by trans-complementation in an F1, or by cis-complementation in an intra-immune response region crossover, new class II products are observed that are not associated with the parental class II proteins. In the chicken the class II MHC genes have also been detected by monoclonal antibody, but at the molecular level sofar only evidence for genes of the β -chain has become available (Bourlet et al., 1988).

Chickens of the Regional Poultry Research Laboratory inbred lines have been extensively studied for disease associations with the B-complex, surface allo-antigens of T-lymphocytes and of B-lymphocytes. Gilmour et al. (1983 and 1986) reported on their regressor ability to Rous sarcomavirus in F4 generation progeny. In the first study the T-cell antigen loci interacted, including homozygous and heterozygous interaction: This was caused by dominance of one locus, but not the other. In the second study interaction was observed between a locus for antigens of peripheral T-cells and allo-antigens of B-cells.

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