

Phenotypic, Genetic and Epigenetic Variation of Immune Response and Disease Resistance Traits of Pigs

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■ Introduction

The immune system is composed of integrated, genetically and environmentally regulated sets of cells and molecules that control the response to external and internal stimuli, including pathogenic microorganisms. In terms of infectious organisms, the response of the host largely reflects the relationship or adaptation between the host and agent. The variation in host response is influenced through genotype by environmental interactions. Epigenetic effects are also highly sensitive to environmental influence and thus can rapidly alter individual phenotype. These are non-sequence alterations to DNA, such as DNA methylation or modification of histones (proteins involved in coiling of DNA), that cause changes in DNA structure that affect its availability for transcription. Consequently, disease is largely the product of incompatible gene by environmental interactions that include both genetic and epigenetic effects. These interactions vary at both the individual and population level. It is therefore particularly relevant to understand host-pathogen relationships and adaptations under various stress and management conditions. Improved understanding of the biology and genetic relationships between the host and pathogen, particularly those that affect the immune system during periods of production stress, should facilitate implementation of non-traditional approaches to improve the health of intensively reared livestock. This paper will briefly describe some of the genetic and environmental variation documented in immune response and performance traits of pigs, mainly using examples from experiments performed by the authors over the last two decades of research.

■ **Current Problems in Animal Health and a Proposed Solution**

Infectious diseases of livestock are currently the most costly and hazardous problem facing the agri-food industry. Costs are estimated at \$18 billion/year in North America. Emerging and re-emerging diseases, many of which are zoonotic, the increasing restriction on antibiotic use and the sizeable costs associated with new drug development are making it more difficult to manage animal health. In addition, consumer concern for both improved food-safety and animal well-being demands an alternative approach for disease prevention that does not rely on extensive use of anti-microbials. One proposed solution that takes advantage of individual variation is to identify and select individuals with advantageous immune response genotypes that have expression in a broad range of environments. Integrating quantitative and molecular genetic strategies to enhance the immune performance and improve inherent disease resistance of pigs, as well as other livestock species, has been the focus of much research. This approach has the ability to advance global competitiveness by lowering production costs and offering superior, high health food products.

■ **Sources of Phenotypic Variation**

The observed performance of an individual for any trait of interest, including immune responsiveness, is known as its phenotype. The two main factors that interact to determine the phenotypic value are genotype and environment (Nicholas, 1987). The genotypic value includes the combined effects of all genes and their interactions, whereas the environment includes the combined effects of all non-genetic factors. The genotypic value (G) of an individual is determined at conception and the environment deviation (E) is the combined effect of all the factors that have influenced the phenotypic value (P) of a particular trait up to the time when the phenotype is measured. This relationship is commonly expressed in the following equation: $P = G + E$, and the genetic component can be partitioned into linear additive (A), dominance (D) and epistatic (the interaction between nonallelic genes - I) fractions, where A is also known as the breeding value and D and I take into account how genes dominate, combine and interact between loci. The descriptive equation therefore can be more comprehensively written as $P = A + D + I + E$, and the proportion of the additive genetic variance in relation to the phenotypic variance (V_A/V_P) is known as heritability (h^2). This information can be used not only to estimate an animal's breeding value (the sum of the effects of all favorable genes), but also to understand why individuals perform in a particular fashion and to select superior individuals for breeding (Nicholas, 1987).

With these concepts in mind, experiments were undertaken to partition the variance associated with immune response traits of pigs and to evaluate the magnitude of influence of particular genes, such as those within the Major Histocompatibility Complex (MHC) that are important in antigen presentation and regulation of immune responsiveness.

Genetic and Other Effects on Immune Response Traits

Miniature pigs with defined MHC genotypes (SLA a/a, c/c, d/d and g/g) were used in initial experiments to evaluate the influence of proposed “major” genes on innate and adaptive host defense mechanisms (Mallard et al., 1989a, b). Genes within the MHC play an important role in antigen processing and presentation. Antibody (AMIR) and cell-mediated immune responses (CMIR) were examined as adaptive features particularly relevant to the control of extra- and intracellular pathogens, respectively. The statistical analyses showed significant effects of MHC, sire, dam, litter, season of birth, parity and gender and the model accounted for ~ 40% and 65% of the phenotypic variation in CMIR and AMIR, respectively (Mallard et al., 1989b). Although the effects were generally greater for sire, dam and litter, the MHC genes contributed about 10% to the observed variation in immune responsiveness. Pigs expressing the SLA d/d and g/g genotypes had the highest AMIR. CMIR also varied by the SLA genotype. The significant influence of litter in this model was mainly environmental, emphasizing the influence of both genes and environment on immune response traits. A population of commercial Yorkshire pigs selected for high and low immune responses also differed in the frequencies of SLA gene expression (**Figure 1**). Not unexpectedly, a large variety of genes associated with variation in host defense have been reported using genomic (e.g. microarray) and other technology (Hammamieh et al 2003, Moser et al 2004; Murtaugh 2006).

Figure 1. SLA Gene Frequencies Differ in High and Low Immune Response Yorkshire Pigs. Gene Identification was by Restriction Fragment Length Polymorphism (RFLP) Patterns at Generation 5 of Selection.

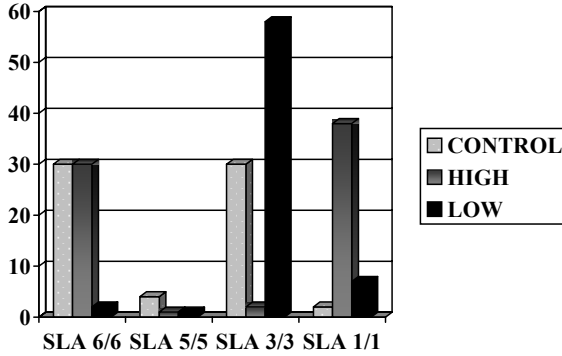


Figure 2. Combined Antibody and Cell-Mediated Immune Response Estimated Breeding Values Ranked Within High, Control and Low Immune Response Lines (Wilkie and Mallard, 1999b).

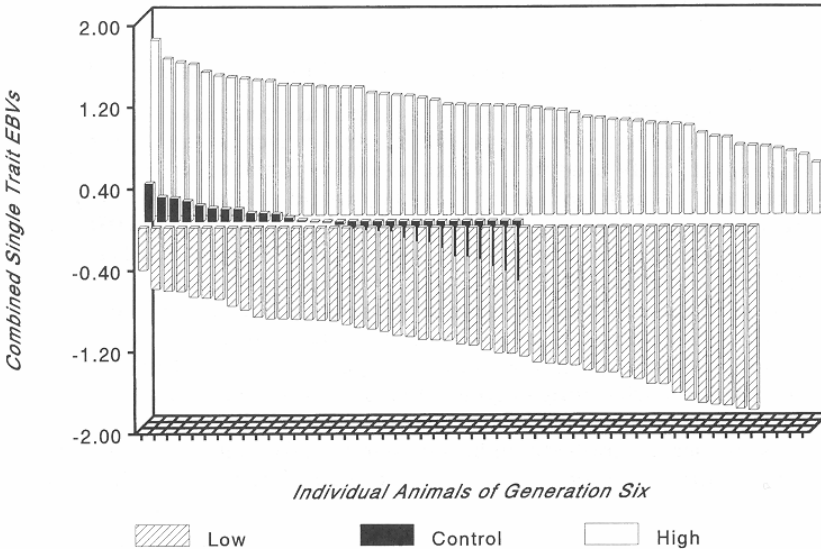
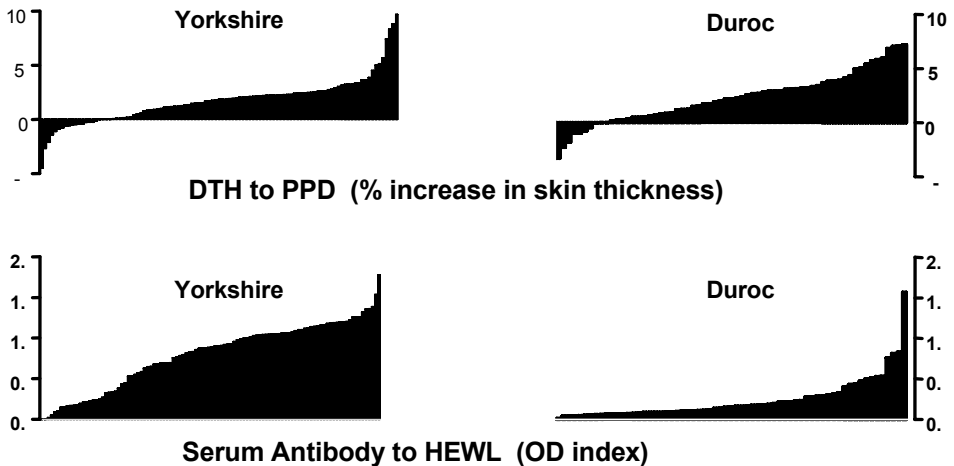


Figure 3. Individual and Breed Variation in Antibody and Cell-Mediated Immune Response Traits of Commercial Pigs (Wilkie and Mallard, 1999b).



Selection of Pigs for High and Low Immune Response

Given that MHC genes only represent a few of the many thousands of genes involved in host defense, it is not an effective strategy to select animals on a gene-by-gene basis in order to improve immune responses or broad-based disease resistance. This approach is only appropriate when one or a few genes are known to control susceptibility; for example, the case of the halothane gene and its effect on malignant hypothermia and PSE pork. It may be possible to produce designer pigs on a gene-by-gene basis at some point in the future when all genes and their interactions are well established. Until then it seems best to approach complex traits, including immune response and disease resistance, from a well-established quantitative animal breeding point of view. To this end, a selection experiment was performed to identify and breed Yorkshire pigs for high and low immune responses based on combined estimated breeding values (EBV) of antibody and cell-mediated immune responses. Results indicated substantial variation between pig EBV (**Figure 2**) and that AMIR and CMIR traits are moderately highly heritable with h^2 values in the range of 0.25-0.35 and 0.12-0.25, respectively (Wilkie and Mallard, 1999). High, low and randomly bred control lines were established over 9 generations of selection and pigs examined for health and performance. High line pigs had consistently higher responses to commercial vaccines, fewer mummified fetuses following a natural outbreak of parvovirus infection, lower overall disease scores following challenge with *Mycobacteria hyorhinus*, and improved rate of gain compared to pigs of the low or control lines (Magnusson et al 1999; Wilkie and Mallard, 2000). No differences in carcass traits were noted between the lines. Similar experiments repeated at a commercial facility indicated substantial phenotypic variation in AMIR and

CMIR between pig breeds (**Figure 3**) and showed that a refined immune response index could be used to identify high and low responder pigs. Variation in immune responses of pigs has been reported by others with h^2 estimates in the same range as reported here (Joling et al., 1993; Edfors-Lilja et al., 1994). Also, others have reported that higher or lower immune responses of pigs were associated with disease outcome. For instance, Xiao et al (2004) noted that weak CMIR responses contributed to prolonged porcine reproductive and respiratory syndrome virus (PRRSV) infection.

Most recently, individual variation in immunoglobulin (Ig) G1 and G2 ratios among high and low responders has been documented, indicative of differences in type 1 (AMIR) and type 2 (CMIR) responses that predominate to control intra- and extra cellular pathogens, respectively (Crawley et al., 2005). Additionally, significant variation in the production of the cytokines (the hormones of the immune system), such as interleukin (IL)-10, IL-12, and interferon (IFN)- γ has been reported among Yorkshire pigs (Crawley et al., 2003). Others have also reported phenotypic and genetic variation in cytokine production of pigs; including mitogen-induced production of IL-2, IL-4, IL-10 and IFN- γ (Edfors-Lilja et al., 1991; de Groot et al., 2005). Pigs infected with various pathogens, such as *Schistosoma japonicum*, *Actinobacillus pleuropneumonia*, leptospira, PRRSV or sarcoptic mange often demonstrate a predominant type 1 or 2 immune response depending on the nature of the pathogen, but that response varies significantly between animals and across breeds (Nguyen et al., 1998; Furesz et al., 1998; Oswald et al., 2001; Lowenstein et al., 2004; Lee et al 2004).

■ Conclusion

These data demonstrate that considerable phenotypic variation exists in immune response traits of pigs and that a substantial portion of that variation is due to additive genetic variance allowing for selective breeding of pigs with higher immune responsiveness. The underlying principle being that optimal disease resistance should be a function of optimal resistance-mediating defense mechanisms. Genes that underlie high or low immune responses and disease resistance are beginning to be understood, such as those within the MHC; however, selective breeding for complex disease traits should still be based on estimated breeding values of superior heritable phenotypes. The next step will be to evaluate high and low immune responsiveness and its influence on health and production traits in several commercial breeding nucleus herds.

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