MOLECULAR ASPECTS OF POULTRY BREEDING

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SUMMARY

A major challenge for molecular biologists interested in animal breeding is the identification of alleles which determine quantitative traits. Chickens are an ideal system for this goal, due to the short generation interval, the existence of genetically well defined strains and their commercial value. Here we describe two approaches towards the identification of quantitative trait genes. The first and most labour extensive approach, is the identification of chromosomal markers, the construction of a gene map and a segregation analysis of quantitative traits with chromosomal markers. The second approach is the identification of alleles which respond to selection. It requires the availability of well defined selected and non-selected strains of chickens and a judicial choice of the chromosomal segments to be analyzed. Three examples are given where the latter approach appears to have been successful. They concern the influence of selection for egg production and/or Marek's disease resistance on the incidence of endogenous viral genes, of a VNTR-locus and of alleles of the proto-oncogene myb.

INTRODUCTION

Recent advances in molecular biology have provided new tools and methods which will have a major impact on animal agriculture. Two areas of research are of particular importance and will revolutionize animal breeding in the near future. These are the development of techniques to identify and characterize genes and the development of gene transfer.

In medicine, an increasing number of genetic disease loci are being mapped by segregation analysis using chromosomal markers, setting the stage for isolating the responsible genes and identifying the gene products (Botstein et al., 1980; Donis-Keller et al., 1987). This provides the necessary basis for early diagnosis, therapy and in some cases cure by genetic engineering. The potential of this approach has spawned the initiation of extensive programs aimed at establishing a complete linkage map of the entire human genome. Similar strategies as developed in medicine can be used for establishing linkage maps of farm animals for usage in marker assisted selection and for ultimately identifying genes or groups of genes which determine quantitative traits (Beckmann & Soller, 1983; Soller & Beckmann, 1983). Such knowledge will provide the bases for selecting of favourable alleles in breeding populations at the DNA level, in the absence of phenotypic expression.

The second area of research of major impact, the transfer of genes into the germline cells of plants and animals, will open the gate to alter the genetic make-up and to create new varieties with higher yields, improved quality, better adaptation to the environment and to permit ecologically more sound farm practices. While gene transfer has already resulted in the development of new plant species, progress in improving farm animals has been slower due to the higher complexity of the organism. However, major breakthroughs in the last year indicate that targeted gene transfer in animals will soon be a reality, thus

offering the possibility to alter specific genes in a predesigned fashion (Westphal, 1989; Cappechi, 1989).

It is more than likely that within ten years linkage maps of all major agricultural animals will have been established and the gene and alleles which affect major production traits mapped, isolated and characterized. This will permit rapid identification of favourable alleles in breeding populations and permit selection without necessitating performance evaluation which is often difficult (e.g. disease resistance) or requires phenotypic evaluation throughout the animals life span (e.g. milk production, egg production). In addition, alleles which are only expressed in the female can also be detected in the male. Within the same time span, targeted gene insertion and mutagenesis will have been developed for these species. This will permit the introduction of new alleles into breeding populations and thus infinitively expand the allelic variations currently available to the breeder.

In our discussion we will restrict ourselves to the first of these research areas, the identification of quantitative trait genes which may be candidates for selection or gene transfer. Even more specifically, we will restrict ourselves to discussing trait genes for which natural alleles exist in breeding populations. The current state of the art of gene transfer will be discussed in other sections of this symposium.

WHAT GENES ARE USEFUL FOR THE BREEDER ?

There are of course numerous genes known which determine "production" ranging from peptide hormones involved in reproduction, differentiation and growth to enzymes involved in metabolic pathways and structural proteins. However, most of these genes have stringently controlled functions throughout the development of the animal and cannot be altered in a way which is beneficial to production. The real challenge therefore is to identify those genes which not only are rate limiting for certain production parameters but can also be altered to improve production traits.

In the near future such information will be gained mainly from physiological studies of specific systems such as nutritional metabolism, muscle growth or the reproductive cycle. Whether enzymes identified through such studies are rate limiting or can be altered in a gainful way can then be studied by adding additional genes, introducing antisense genes or by site specific mutagenesis (Walter, 1988; Cappechi, 1989). In the case of peptide hormones, such information can be directly obtained by injection of the hormone or hormone antibodies.

Such physiological studies will be discussed in other sections. Here we will discuss another approach which relies on identifying of genes for which natural allelic variations exist which affect quantitative traits. This approach will not only lead to the identification of genes which are rate limiting for the particular trait, but a detailed analysis of the alleles will lead to the elucidation of what types of mutational changes affect the trait. It will provide clues on how to further manipulate such genes in order to create new alleles.

APPROACHES FOR IDENTIFYING QUANTITATIVE TRAIT GENES

(A) SEGREGATION ANALYSIS

The most general approach towards identifying genes which affect productivity is the development of a linkage map of the chicken genome and the search for linkage of production traits with genetic markers. It entails the mating of two widely divergent strains of chickens, the establishment of F2 progeny and a linkage analysis between chromosomal markers and quantitative traits. This requires the identification of chromosomal markers which distinguish the alleles present in the original mating partners and the screening of the F2 progeny for as many traits as possible (White & Lalouel, 1988).

An abundant source of genetic markers that are useful for segregation analysis are base changes in DNA which occur at sites recognized by restriction endonucleases and alter the lengths of restriction fragments obtained upon digesting genomic DNA (restriction fragment length polymorphisms). An additional class of polymorphisms is provided by minisatellite DNA's. These loci are called "variable number of tandem repeat loci" (VNTR-loci) since they give rise to polymorphisms due to variations in the number of tandem repeats of a core sequence (Jeffreys, 1987; Nakamura *et al.*, 1987).

Another set of markers are DNA sequences related to the avian leucosis virus (*ev*-genes). More than 20 different *ev*-genes have been identified in the chromosome of White Leghorn chickens and many more are present in other breeds of chickens. Several *ev*-genes have been mapped to chromosomes by *in situ* hybridisation and will provide useful references when establishing linkage maps (Rovigatti & Astrin, 1983; Smith, 1986).

A new class of *ev*-genes has recently been identified. They are not closely related to the avian leucosis virus, but have the typical structure of a retrovirus. They occur in the genome at a high frequency and might provide additional useful probes for chromosome mapping (Dunwiddie *et al.*, 1986).

Several reference crosses to conduct segregation analyses are being prepared by the Animal Research Centre of Agriculture Canada, Ottawa (Gavora, personal communication) and the performance of each individual bird is monitored. A repository of DNA samples of these birds is being established and will be accessible for future linkage analysis.

(B) IDENTIFICATION OF ALLELES WHICH RESPOND TO SELECTION

A more restricted but powerful approach towards identifying trait-associated genes is to search for genes which have alleles which respond to selection. It entails the identification of co-selected genetic markers in well defined differentially selected strains of poultry and the analysis of adjacent genes.

This approach is more restricted than identifying quantitative trait genes by segregation analysis, since only very tight linkages are preserved during selection. Rather than searching for co-selection of random genetic markers with quantitative traits, DNA polymorphisms in specific chromosomal regions which might be directly involved in determining quantitative traits have to be analyzed.

Table 1: Frequency of ev-genes in strains selected for egg production traits and non-selected control strains.[1]

ev-locus	Phenotype[1]	Genetic base I strains			Genetic base II strains		
		7 (N=33) control	8 (N=37) selected	9 (N=35) selected	5 (N=33) control	3 (N=37) selected	1 (N=35) selected
1	gs^-chf^-	1.00	.97	1.00	.97	1.00	1.00
4[3]	gs^-chf^-	.09	.49[4]	.43[4]			
5	gs^-chf^-	.58	.46	.43			
8[3,5]	gs^-chf^-	<.03	.19[4]	.14[4]	.03	.20[4]	.14
15	gs^-chf^-				.03	.80[4]	.76[4]
6	gs^-chf^+	.48	.54	.66	.42	.59	.48
9[3]	gs^-chf^+	.27	.08[4]	.08[4]			
3	gs^+chf^+	.61	.46	.57	.69	.59	.52
18[3]	$V\text{-}E^+$				.44	<.03[4]	<.03[4]
7[3,5]	unknown	.06	.30[4]	.17			
new C[3]	unknown				.19	<.03[4]	<.03[4]

[1]Strains are White Leghorn's kept non-selected (7,5) or selected for egg-production and related traits (8,9,3,1). See Legend to Fig. 2 for further details.
[2]Summarized by Smith (1986). Phenotypes are abbreviated as follows: chf^+, expression of the viral envelope protein; gs^+, expression of internal viral gag-proteins; $V\text{-}E^+$, production of infectious endogenous virus.
[3]Ev-genes whose frequency varied significantly among the three strains of the respective set (chi-square test for three independent samples, $P<0.05$).
[4]Significantly different from the corresponding control strain (chi-square test of Fisher's exact probability test, $P<0.05$).
[5]The pooled frequencies of the selected strains are significantly different from the frequency in the non-selected control strain (Fisher's exact probability test, $P<0.01$).

Candidates for such chromosomal regions are genes suspected to directly determine productivity, hypervariable minisatellites and DNA segments related to the endogenous avian leucosis virus (ev-genes). The rationale for including analyses of polymorphisms in hypervariable minisatellite regions and ev-genes are as follows. Some hypervariable minisatellite regions are hot-spots of gene conversion and meiotic recombination and might therefore mark chromosome regions which give rise to allelic variations (Uematsu et al., 1986, Jarman & Wells, 1989). Ev-genes, on the other hand, have strong promotors which may enhance the expression of neighbouring genes (Cullen et al., 1984). Further, they may create phenotypic variability by integrating within a gene and disrupting its function.

EXAMPLES OF ALLELES WHICH RESPOND TO SELECTION

(A) ENDOGENOUS VIRAL GENES

Endogenous viral genes (ev-genes) are - with the possible exceptions of MD resistance alleles in the major histocompatibility locus - the only genes for which natural alleles have been identified at the DNA level (i.e. presence or absence of the ev-genes) which affect quantitative traits. Ev-genes are genes related to the exogenous avian leucosis virus. Such genes may be silent, express viral proteins or even complete infectious virus. Based on chromosome location and gene structure, more than twenty different ev-genes have been identified in the White Leghorn alone and many more appear to be present in broilers and other breeds of chickens (Rovigatti & Astrin, 1983; Smith, 1986).

Ev-genes may influence quantitative traits via the biological properties of their gene product. In particular, it has been shown that those ev-genes which express viral envelope proteins render chickens more susceptible to infection by exogenous avian leucosis, presumably because they induce immune tolerance (Crittenden et al., 1982; Crittenden et al., 1984). Alternatively, they may be integrated within a gene and disrupt its function. This may be the case with ev-21, a virus producing gene which is located on the male (Z) chromosome and is responsible for the slow-feathering trait used in feather-sexing (Smith & Crittenden, 1986). The loss of the slow feathering trait has been shown to be associated with the loss of ev-21, while the loss of ev-21 leads to the loss of the slow feathering trait. The mechanism by which ev-21 leads to slow feathering is not known. It has been proposed that the integration of ev-21 may directly affect the expression of a putative "slow feathering" gene, although the possibility that the endogenous virus produced in the rapidely dividing cells of the feather pulp may interfere with feather growth cannot be excluded.

In our laboratory we have analyzed the influence of selection for egg production and related traits on the incidence of ev-genes in two sets of strains, each consisting of a control and two duplicate selected strains (Kuhnlein et al., 1989). Selection for egg production traits reproducibly affected the frequency of ev-genes in each duplicate selected strain, indicating the absence of random genetic drift (Table 1).

All but one of ev-genes which were co-selected with production traits have deletions of the 5' viral promotor (LTR). Such deletions activate, in many instances, the transcription of genes down-stream of the viral insertion site, starting from the usually inactive 3' LTR of the endogenous viral gene (Cullen et al. , 1984). It raises the possibility that over-expression of the flanking

Table 2: Frequency of the endogenous gene ev-6 in strains selected for MD resistance and in non-selected strains:

Genetic base	Not selected for MD resistance		Selected for MD resistance	
	Strain	Ev-6 frequency	Strain	Ev-6 frequency
Base I	7	0.48	8R	0.25
	8	0.54	WG	<0.10
	9	0.66	WD	<0.14
Base II	5	0.42	3R	0.21
	3	0.59		
	1	0.48		
	GH	0.42	GF	<0.04

Strains were derived from four North American commercial stocks of White Leghorns (base I) and from a narrow base of stocks at Agriculture Canada (base II). Strains 7 and 5 were kept non-selected since 1958 and 1950, respectively. Strains 8, 9, 3 and 1 were selected for egg production and related traits. Strain 8R was derived from strains 8 and 9 by first deriving inbred lines, selecting for MD resistance and intermating the best ten lines. Strain 3R was derived similarly from strains 3 and 1. WG and WD are MD resistant inbred lines derived from strain 8. GH and GF are inbred lines derived from strain 3 and are susceptible and resistant to MD, respectively (Gavora et al., 1982).

genes located 3' to these ev-genes confer a selective advantage. Characterization of the flanking region or the putative transcripts starting at the 3' LTR's might therefore lead to the indentification of genes whose expression is rate limiting.

The endogenous viral genes which were selected against, were genes which express parts of endogenous virus or complete infectious virus. Such genes may affect production traits via their gene product by some as yet not characterized interactions of their gene products with physiological processes. The negative affect of such genes has recently been directly demonstrated by developing a semicongenic line which segregates for ev-12, an ev-gene which codes for infectious endogenous virus. Chickens carrying this gene had a reduced ovulation rate, egg weight and shell thickness (Gavora et al., unpublished).

In addition to strains selected for egg production we have analyzed strains and lines derived from the same genetic basis as above, but selected for Marek's disease (MD) resistance. As shown in Table 2, all Marek's disease resistant strains or lines were marked by a reduced incidence of ev-6, indicating that this ev-gene may be associated with MD resistance.

Ev-6 is an endogenous viral gene which is transcribed despite the absence of a viral promotor and is therefore thought to be under the control of a host cell promotor. It is highly expressed in plasma cells, indicating that it is integrated in a gene which is usually expressed in these cells (Ewert & Halpern, 1982). This gene may therefore be associated with the humoral immune response and, when disrupted by ev-6, lead to MD susceptibility.

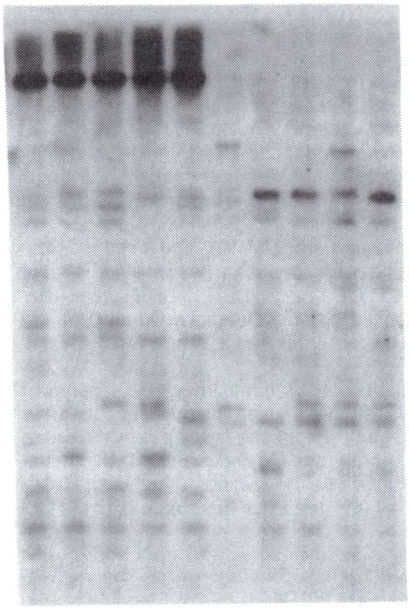
(B) VNTR-LOCI

Variable tandem repeat (VNTR) loci or hypervariable minisatellite loci are DNA segments which consist of tandem repeats of relatively short DNA sequences and give rise to a relatively high frequency of alleles due to variations in the number of repeats (Jeffreys, 1987; Nakamura et al., 1987). Based on sequence similarity minisatellites at different loci can be divided into families. In Southern blot a single probe will therefore often hybridize to several loci giving rise to a banding pattern called a DNA fingerprint. DNA fingerprinting can be applied to poultry to measure inbreeding and genetic distances between breeding populations (Kuhnlein et al., 1989 & 1990).

When analyzing two White Leghorn strains, S and K, using M13 as a DNA fingerprinting probe we noticed the presence of two closely migrating bands of about 25 kb in strain K (Fig. 1). These bands were several fold more intense than any other bands present in either strain S or K. Segregation analyses indicate that these dominant signals represent two alleles of a locus composed of tandem repeats of a DNA segment which cross hybridizes with the DNA fingerprinting consensus sequence of M13 (Vassart et al., 1987).

Strains S and K are White Leghorn strains derived from a common genetic base and selected for susceptibility and resistance to MD, respectively (Hutt & Cole, 1957). In order to test whether these tandem repeat sequences were associated with MD resistance we analyzed a second set of strains consisting of strains 8 and 9 (not selected for MD resistance) and 8R (derived from strains 8 and 9 and selected for MD resistance). An amplified DNA segment was present in all birds of strain 8R, but only in 60% of the chickens of strain 8 and 9. Moreover, many of the birds in the latter two strains had only one band rather

Figure 1: Co-selection of a VNTR locus with MD resistance



The left five lanes are DNA fingerprints of chickens from the MD resistant strain K and the right five lanes DNA fingerprints of chickens from the MD susceptible strain S. Both strains had been derived from a common genetic base at Cornell in 1936 and were kept as closed breeding populations with the exception of a few Kimber birds which were intermated with strain K in 1936 and 1940 (Hutt & Cole, 1947).

than two, indicating the presence of a single allele carrying an amplified DNA segment. In addition, in two highly inbred lines 6_3 and 7_2, derived by the Regional Poultry Research Station of the USDA at East Lansing, Michigan, and differing in MD susceptibility, a highly amplified DNA segment was observed in the resistant but not in the susceptible strain (Stone, 1975; Kuhnlein et al., unpublished).

We are currently analyzing the effect of selection for egg and meat production traits on the incidence of specific VNTR loci. It will provide us with an estimate of number of genes which have alleles affecting these more complex traits.

(C) SELECTION FOR ALLELES IN GENES IMPLICATED IN DETERMINING PRODUCTION TRAITS

A third approach is to search for alleles in genes known to be determinants of quantitative traits. We have applied this approach to the myb proto-oncogene, a gene that has been shown to be involved in blood stem cell differentiation and perhaps in other differentiation pathways as well (McMahon et al., 1988) Particularly high levels of expression have been observed in the thymus, suggesting that it may play an important role in T-cell differentiation (Thompson et al., 1986). Since T-cells are the target cells for transformation by MD virus (Hudson & Payne, 1973) an altered myb gene might affect the resistance to MD by changing the kinetics of T-cell differentiation and hence the number of target cells.

Screening different strains of chickens using the MspI restriction enzyme, revealed the presence of two different polymorphisms in the myb gene, both located in the 3' half of the gene. Analysis of strains 8, 9 and their MD-resistant derivative 8R, revealed co-selection of one of the two polymorphisms in strain 8R, whereas the other polymorphism was neutral. Analysis of two other lines, GH and GF, both derived from a common ancestral strain of White Leghorns and differing in MD resistance also revealed a higher frequency of this polymorphism in the resistant line.

Whether this polymorphism reflects an altered myb protein, an altered regulatory control of the myb gene transcription or simply an RFLP linkage with another gene remains to be determined. However, differences in the methylation of the polymorphic MspI site among tissues indicates that the RFLP might directly affect MD resistance.

CONCLUSION

In this paper we presented some approaches for identifying genes which affect productivity traits. Although, the most complete approach is the establishment of linkage maps and the identification of linked trait genes by segregation analysis, this approach is very labour intensive and costly. An alternative approach is to search for alleles which respond to selection. By definition, such alleles are rate limiting and therefore good candidates for future manipulation by genetic engineering or can be used for selection at the DNA level. This approach is dependant on having well defined differentially selected strains which have been kept as closed breeding populations and whose effective population sizes are large enough to minimize genetic drift. We highly encourage the developement and maintenance of such strains.

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