ANALYSIS OF GENETIC ASSOCIATION BETWEEN DNA FINGERPRINT BANDS AND QUANTITATIVE TRAITS USING DNA MIXES

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SUMMARY

The use of multilocus probes as genetic markers for linkage analysis is limited in non-prolific animals, such as dairy cattle, due to the relatively high levels of band sharing between unrelated individuals. We propose to overcome this difficulty by fingerprinting DNA mixes of groups representing the distribution of a quantitative trait. This approach was tested on a segregating population of broilers, regarding abdominal fat deposition. Two DNA fingerprint bands associated with abdominal fat deposition were identified. Correlation between frequency of band P6.1 and fat percentage is highly significant (r=-0.93) and its effects on fat percentage and fat weight are -0.24% and -4.44gr, respectively.

INTRODUCTION

DNA markers, such as RFLPs and DNA fingerprints (DFPs) are available at any developmental stage, in any tissue and in both sexes. Their co-dominance mode of inheritance enables to distinguish the heterozygote from both homozygotes, and makes them suitable to be used as genetic markers (Beckmann et al. 1983, Hill 1987, Hillel 1989a, 1989b, 1990, Jeffreys et al. 1987, Soller et al. 1986). DNA fingerprints loci are highly polymorphic with extremely high levels of heterozygosity, and therefore, are advantageous for studying the association between molecular markers and quantitative traits. A procedure to identify DFP bands linked to major genes controlling quantitative traits, and its application in chickens, have recently been described (Hillel et al., 1989a, 1989b, 1990).

Application of DFP for linkage analysis in animals of low prolificacy such as cattle, is restricted due to relatively high level of band sharing (≈25%) between unrelated individuals (Jeffreys et al., 1985; Hillel et al., 1989). The required large sibship of such animals is usually composed of many unrelated dams. Consequently, analysis of paternal DFP bands in a large paternal half-sib family, would leave negligible number of bands for analysis due to band sharing between the sire and the many dams. Furthermore, dam bands are not useful for linkage analysis within full-sib families due to the small number of offspring per dam.

In this paper a possible solution for the shortcomings of DFP linkage analysis in non-prolific animals is presented.

THE PROPOSED APPROACH

We propose to compare DFPs of mixes of DNA from high and low performing daughters of one sire, rather than to analyze DFPs of the individual animals. DFP patterns of these mixes will contain the paternal bands and non-polymorphic bands shared between the sire and most of the dams. Bands present in few dams will not appear on the radiogram due to their low frequency in the mix.

In the case of dairy cattle, the massive use of artificial insemination produces very large paternal half-sib families. An appropriate family to be analyzed by the proposed approach should include several thousands daughters of one sire, with phenotype information regarding economically important quantitative traits, adjusted for herd-year-season factors and averaged over lactations. As the analysis is carried out on paternal bands, it is preferable to biometrically remove the genetic merit of dams and dams' relatives from daughters' records. Due to the large family size, sufficiently large number (about 100) of daughters can be selected at high selection intensity ($P < 10\%$) at each side ("tail") of a quantitative trait's distribution. This approach is therefore named "Tail Selection" (TS). For each of these two groups, equal amounts of DNA from each individual are mixed. The DFP patterns of the two mixes are expected to be very similar, having the same paternal bands and non-polymorphic bands common to the sire and dams. Difference between the two patterns, i.e., bands specific to one "tail" only or significant difference in band intensity, are attributable to linkage between these specific bands and major genes affecting the quantitative trait.

Whenever possible, it is advised to analyze not only the two extreme groups, but to represent the entire family by several groups along the distribution of the quantitative trait. Given that this distribution is of genetic origin, these groups represent different frequencies of the desired alleles controlling the trait. Therefore, a gradient of band intensity among the different groups (high intensity in one extreme group decreasing towards the other extreme group) can be interpreted as an association between this band and the analyzed quantitative trait. This gradient approach is especially useful when the difference in band intensity between the two extreme groups is relatively small.

MATERIALS AND METHODS

Although dairy cattle is an ideal population for the proposed approach, this presentation deals with broilers due to availability of the birds and their DNA. The sire used was a male offspring of a cross between low fat (LF) and high fat (HF) lines (Cahaner, 1988). This sire was crossed with three LF dams to produce a segregating BC1 generation. Sixty five offspring were analyzed. The number of birds analyzed in each of the three dams were 13 (Dam 1), 21 (Dam 2) and 31 (Dam 3). At six weeks of age, body weights (BW) and abdominal fat weights (FW) were recorded, and abdominal fat percentage (F% = FW*100/BW) was calculated. Individual offspring were sorted by their F% and divided to seven sub-groups within each sex and dam-family combination. These sub-groups were grouped over sexes and dam families according to their rank, dividing the entire sire-family to seven groups, from the leanest, averaged 0.46% F%, to the fattest, averaged 1.56% F% (Table 1).

DNA mixes were prepared from each group, and digested by HinfI restriction endonuclease. DNA fingerprints of these mixes were prepared according to Hillel et al. (1989a), using Jeffreys probe 33.6. Two DFP replicates of the seven groups were prepared on one gel, one from the leanest group to the fattest and a second in the backwards order. This allowed comparisons free of a band intensities gradient from one side of the gel to the other, due to technical artifacts. Individual DFPs of the 65 birds and their parents were also prepared and analyzed.

RESULTS

Eleven paternal bands were evaluated for band-intensity gradient. Two paternal bands were found to show such a gradient: high intensity in the group of lean birds and a decrease towards the fat birds group. Results concerning one of these bands, designated P6.1, are presented in this paper. Based on this gradient of intensities, all 65 birds were analyzed regarding band P6.1. As a result, frequency of P6.1 in each of the seven groups was obtained (Table 1).

Table 1 - Frequency of P6.1 carriers and mean fat percentage (F%) by group

	Group number						
	1	2	3	4	5	6	7
Birds/group	9	10	9	9	8	10	10
F% mean	.46	.65	.81	1.03	1.19	1.36	1.56
S.E.	.05	.05	.06	.06	.09	.09	.10
P6.1 carriers							
Number	8	8	7	5	3	5	4
Frequency	.89	.80	.78	.55	.37	.50	.40

Association between band frequency and fat percentage was determined by simple linear correlation ($r=-0.93$, $P(r)=0.005$). The distributions of males and females and representation of the three dams in each of the seven groups were homogeneous, therefore, no adjustments were needed prior to the grouping.

The effect of band P6.1 on each of the three quantitative traits (F%, FW and BW), was estimated by multiple regression analysis using data of the 65 individuals. Adjustments for both sex and dam were included in this regression analysis. Population means and effects of band P6.1 on each of the three quantitative traits are given in Table 2, taking the dam and sex effects into consideration.

Table 2 Effects of P6.1 on fat weight (FW), fat percentage (F%) and body weight (BW). P is the significance level of the estimate.

Trait	Mean	Est. b SE	P
F% (%)	1.02	-0.24 þ .1	.02
FW (g)	17.3	-4.44 þ 1.98	.03
BW (g)	1662	-30 þ 68	NS

As was expected from the intensity gradient on the DFP autorodiograms and from the associated band frequency, the band effect on fat percentage is highly significant. In addition, the association of the band P6.1 with fat weight was also significant, while association with body weight was not significant.

DISCUSSION

An approach is presented by which the application of multilocus probes to linkage analysis in cattle and other non-prolific farm animals. Using this approach we have identified a DFP band associated with abdominal fat percentage and content in broilers. The effect of this band (P6.1) on abdominal fat percentage and fat weight is substantial (≈ 25% of the population mean) and is independent of body weight as reported earlier (Cahaner 1988). If further examinations will prove the genetic linkage between band P6.1 and major genes controling abdominal fat deposition in broilers, it will be isolated and

purified to be used as a locus specific probe (Wong et al. 1986) for intra-family selection. It is essential to note that the associated band could have been identified by linear model analysis, using the quantitative trait as dependent variable and the DFP bands of each individual as dummy independent variables. The DNA mixes and the gradient approach were used to demonstrate their usefulness to detect bands linked to genes of interest with much less laboratory efforts. This approach is applicable to organisms where large half-sib families are available (e.g., cattle, horses, sheep, goats and fish) or less preferably, to full-sib families of moderate size (e.g., poultry and swine). Therefore, the use of "mixes" DFP converts the limitations of large half-sib family structure to advantage compared to moderate size full-sib families.

In paternal full-sib families, where association between average paternal haplotypes and average performance of the sire is evaluated, the dams' genetic contribution should be omitted by taking into consideration her own repeated performance, her ancestors and lateral relatives genetic merit. It is obvious that analysis of each additional trait would require the establishment of its own distribution for the construction of DNA mixes. When correlated traits, such as milk production and milk protein production ($r_G \geq 0.8$) are analyzed, the extreme tails of the two trait distributions will overlap and, therefore, DNA mixes for both traits could be prepared mostly from the same individuals.

Finally, it should be mentioned that so far only few multilocus probes are available for cattle, although recently there are some improvements (Vassart et al. 1987, Buitkamp et al. 1989, Haberfeld et al. unpublished data). The current situation with sheep (Haberfeld et al. unpublished data), goats (Hillel et al. 1990) and particularly poultry, as shown in this paper, allows intensive studies using procedures similar to the one presented in this paper. The approach presented in this study is currently being tested on dairy cattle at our laboratory. Mixes of DNA are being used for line characterization, assignment of DFP bands to chromosomes and construction of evolutionary trees.

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