GROWTH HORMONE RFLP'S: POTENTIAL GENETIC MARKERS FOR BODY WEIGHT IN MICE

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SUMMARY

Two F_2 populations of mice representing diverse genetic backgrounds were generated from crosses between long-term high 42-day body weight selected and control lines of mice. Haplotypes for restriction fragment length polymorphisms (RFLP's) at the growth hormone (GH) locus, GH^n and GH^c, were identified for approximately 150 mice in each F_2 populations and analyzed to determine the effect of haplotype on body weight.

The two populations yielded apparently contradictory results. In one of the populations, the GH^n allele which was fixed in the selected P_0 was associated with lower 42-day weight, as we found in a previous study with a different population. In the other set, the GH^n allele which was present at a frequency of 0.60 in the selected P_0 was associated with higher weights in the F_2; however, these results were confounded by a dearth of ST^nST^n homozygotes and the presence of heterotic effects on weight.

INTRODUCTION

The potential for utilizing genetic or biochemical markers in determining genotypic values of production animals has long been recognized. The major advantage of including such information derives from the calculation of an individual's genotypic value using its own genotype rather than estimating the average effect of the entire genome from a population of individuals.

In the past, research has focused on revealing genetic correlations between protein polymorphisms and quantitative traits. For example: relationships between bovine major histocompatibility class I antigens and susceptibility to internal parasites, lymphocytosis response to bovine leukemia virus, and early growth traits have been documented (Stear et al., 1988a; 1988b; 1989); Oddgeirsson et al. (1988) looked at bovine major histocompatibility complex erythrocyte markers and mastititis susceptibility; Erhardt and Senft (1989) and Geldermann et al. (1985) have studied correlations between milk protein variants and milk production traits; and Rizzi et al. (1989) focused on blood genetic markers and production traits in sheep. Other studies have been conducted to examine the relationships between hormone profiles and production traits (e.g. Arbona et al. ,1988; Peel and Bauman, 1987; Grigsby and Trenkle, 1986; and Dodson et al., 1983). Promising results attained in these areas suggest the possibility of utilizing genetic polymorphisms for estimating genetic potential.

Advances in molecular genetic techniques have facilitated the detection of genetic polymorphisms at the DNA level . It is now possible to search for correlations between DNA or RFLP's and production traits using either a battery of random genetic markers (reviewed by Tanksley et al., 1989) or a limited number of selected genetic markers. Our research has taken the latter approach, focusing on somatotropic genes and growth traits in mice.

Initial studies carried out in our laboratory (Salmon et al., 1989) examined RFLP's for GH in three lines of mice (2H, 3H and 5H) which had undergone long-term selection for high 42-day weight and their randomly mated control lines (2C, 3C and 5C). This study revealed that polymorphisms existed at the GH locus for all

of seven restriction enzymes studied. One of the haplotypes at this locus, designated GH^n, was fixed in two of the selected lines (3H and 5H) while the other haplotype, designated GH^c, was fixed in all of the control lines. Further research examining the relationship between growth hormone haplotypes and 42-day body weight in an F_2 population generated by crossing the 3H selected line to its appropriate control, 3C, indicated the existence of a significant genetic covariance for weight and haplotype (Winkelman et al., 1990). However, the effect of the high line haplotype on weight appeared to be negative, since GH^cGH^c mice were heaviest and GH^nGH^n mice the lightest. The research reported here was undertaken to assess the effects of these haplotypes in F_2 populations of mice derived from the remaining two selected and control lines. Our purpose was to study the GH haplotype in the diverse genetic backgrounds of populations with similar selection histories.

EXPERIMENTAL METHODS

Mouse Stocks

The mouse populations we are working with were obtained by the University of Alberta from the Lacombe Experimental Station in Alberta. Mice used in this experiment were from two base populations; one of these, 5, was derived from the Q strain obtained from Edinburgh in 1961 and the other, 2, was created by intercrossing four outbred strains (Q, NB, LX, and JH) also brought from Edinburgh in 1961. Each base population has been divided into two lines, a long term selected , H, bred for high 42-day body weight and an unselected control line, C.

Breeding Scheme

The mating scheme which was utilized is shown in Figure 1. The design shown was applied to both the 5Hx5C and 2Hx2C crosses. All matings were single pair matings and all litters were culled to five offspring seven days after parturition. This scheme was designed to circumvent the confounding effects of small base population size and litter size encountered in our previous study. Sib-sib matings were avoided throughout the experiment. This plan created thirty F_2 families, each of five offspring, for analysis.

Data Collected

All mice from P_1, F_1, and F_2 generations were weighed at 21, 28, 35 and 42 days of age.

The F_2 mice were sacrificed after the last weighing, livers were dissected out, frozen immediately in liquid nitrogen and stored at -40°C. Genomic DNA was extracted from individual livers and digested with Hind III in the presence of 5mM spermidine. Restricted DNA samples were fractionated on 0.7% (w/v) agarose gels and transferred to Gene Screen Plus (NEN) membranes. Membranes were hybridized with a ^{32}P-oligolabeled rat growth hormone cDNA (Seeburg et al., 1977) probe overnight, washed and exposed to Kodak XAR films. From these films, growth hormone RFLP patterns for each mouse were obtained.

The analysis performed to determine the nature of the relationship between growth hormone RFLP's and 42-day live weight, measured as the deviation from the midparent, was carried out separately on males and females for each F_2 population.

RESULTS AND DISCUSSION

Mean weights for P_1, F_1 and F_2 mice are shown in Table 1. The 5H line was significantly ($P<0.05$) heavier than the 5C line for both males and females. Differences between H and C lines in the (2) population were no longer significant

at the onset of this study, which followed 42 generations of random mating since selection was relaxed. It should be noted here that the GH^n haplotype is fixed in the 5H line and the GH^c haplotype is fixed in both the 2C and 5C lines while 2H line is polymorphic with respect to this locus. It is also notable that both of the (2) lines are heavier than the 5H line. This observation is of particular interest in light of our observations utilizing the (3) population, an amalgam of four inbred lines. These indicated that the GH^c haplotype was associated with heavier weights in the F_2 generation (Winkelman et al.,1990). Another distinguishing feature of the (2) population is that heterotic effects for 42-day weight were evident in the F_1 (Table 1) but were not observed in the $5F_1$ or in the $3F_1$ studied previously.

Allele frequencies for GH^n in the (5)F_2 and (2)F_2 populations were 0.48 and 0.30, respectively. As stated above, these allele frequencies are representative of the genetic input from the foundation P_0 populations utilized. The results of a preliminary analysis showing the mean 42-day weights of the three genotypes at the growth hormone locus are given in Table 2. We have found that the results for the $5F_2$ population are essentially the same as those which were observed in our previous study; namely, that the ST^c haplotype which was fixed in the original control population came to be associated with higher weight. This observation suggests that the effect observed in our first study (Winkelman et al.,1990) was not due to a chance recombination event between the growth hormone locus and a closely linked marker affecting size. This would have confounded the results because of the very small foundation population size used in that study. However, the results with the data set on the (2) lines differ. Several factors may have contributed to these results. We have already alluded to the fact that the 2C line is no longer smaller than 2H and that GH^c is present in 2H. Secondly, the analysis of this data was confounded both by the dearth of ST^nST^n homozygotes observed (Table 2) and by heterotic effects (Table 1). Further studies are currently under way to assess the effect of the ST haplotypes in other genetic backgrounds. Analyses of GH haplotypes in 2Cx5H F_2 mice and in selected lines derived from $2F_2$, $5F_2$ and $2.5F_2$ are proposed to test the hypothesis that the GH haplotypes have potential value as parameters in selection indices for weight in mice. We are also examining interactions between polymorphisms at different somatotropic loci.

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Figure 1. Mating scheme utilized to generate populations of F_2 mice from selected by control line crosses

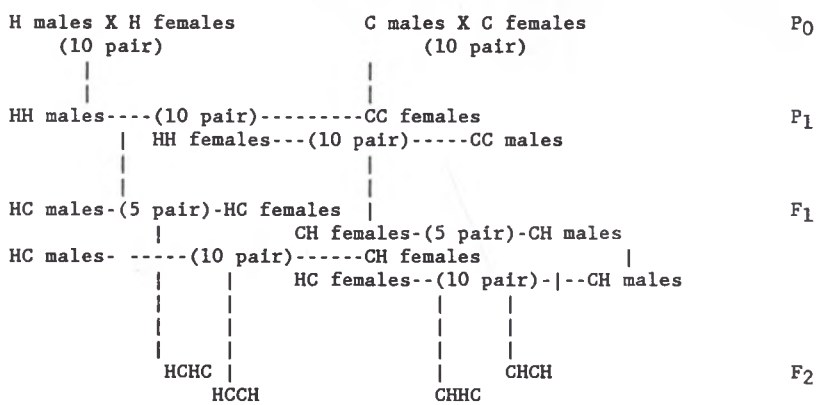

Table 1. Mean 42-day weights (in gm) of P_1, F_1 and F_2 mice.

LINE	n	MALE	n	FEMALE
(5)C	22	28.15±0.08	25	24.11±0.06
(5)H	21	33.83±0.14	17	27.89±0.15
(5)F_1	52	31.93±0.04	41	26.54±0.05
(5)F_2	79	32.81±0.03	68	27.48±0.03
(2)C	24	35.87±0.21	12	29.37±0.13
(2)H	25	37.66±0.12	17	30.03±0.10
(2)F_1	50	40.41±0.07	44	31.00±0.06
(2)F_2	77	41.48±0.04	70	32.02±0.04

Table 2. Mean 42 day weights (in gm) for the three somatotropin RFLP genotypic classes of mice in F_2 populations from high line by control line crosses. Weights were measured as deviations from midparent values.

GENOTYPE	n	MALES	n	FEMALES
(5)				
ST^hST^h	20	2.90±0.13	12	-2.30±0.16
ST^hST^c	37	3.51±0.09	26	-1.88±0.08
ST^cST^c	12	3.69±0.21	26	-1.73±0.11
(2)				
ST^hST^h	6	9.08±0.22	3	0.87±1.69
ST^hST^c	40	6.47±0.09	21	-3.41±0.15
ST^cST^c	30	5.42±0.11	33	-4.37±0.11