Theor Appl Genet (1993) 85:931–936

THEORETICAL AND APPLIED GENETICS

Identification of growth hormone DNA polymorphisms which respond to divergent selection for abdominal fat content in chickens

N. Fotouhi, C. N. Karatzas, U. Kuhnlein, and D. Zadworny

Department of Animal Science, McGill University, Ste. Anne de Bellevue, Quebec H9X 3V9, Canada

Received July 9, 1992; Accepted July 30, 1992
Communicated by C. Smith

Summary. Two strains of meat-type chickens which had been derived from the same genetic base, but were selected for high or low abdominal fat content, respectively, were analyzed for polymorphisms in the growth hormone gene (GH). A total of four DNA polymorphisms were identified, one at a *SacI* restriction site and three at *MspI* restriction sites. Restriction mapping indicated that all polymorphisms were in exons and/or introns and not in flanking regions of the gene. The incidence of GH polymorphisms was determined in 20 chickens from each strain and significant differences were observed for two of the four polymorphisms. Analysis by DNA fingerprinting using $(CAC)_5$ as a probe indicated that the inbreeding coefficient was 0.1 in both strains and that random genetic drift was minimal. Thus, the selection for abdominal fat appears to have affected the frequency of alleles of the growth hormone gene. Whether this is the direct consequence of an altered growth hormone gene on fat metabolism or reflects linkage to an allele of a neighbouring gene remains to be determined.

Key words: Divergent selection – Broiler chickens – Growth hormone gene polymorphisms – Restriction enzyme map – DNA fingerprinting

Introduction

Fatness in broiler lines is undesirable for both the consumer, who is interested in reducing dietary fat, and the producer, who is interested in reducing feed costs and waste. The response to selection based on the size of the abdominal fat pad or on plasma lipoprotein (VLDL) indicates a high heritability of fatness or leanness, respectively (Leclercq 1988; Whitehead 1988). Comparison of divergently selected lines indicates that selection influences lipid metabolism. Notably, Leclercq (1988) reported that, in comparison to the lean line, chickens from the fat line showed increased de-novo lipogenesis in the liver, had elevated concentrations of plasma VLDL and HDL, plasma triglyceride and plasma phospholipid, and an increased number and volume of adipocytes.

One of the hormones which has been shown to influence lipid metabolism in mammals is growth hormone (GH) which appears to reduce lipid accretion and increase lipid mobilization (Breier et al. 1989). There is evidence of a similar action of GH in avian species, but interpretations are complicated by nutrition, age, and species-related effects (Scanes 1987). However, in-vitro experiments indicate that GH increases lipolysis in chicken adipose tissue, indicating that GH might directly affect lipid metabolism at the cellular level (Harvey et al. 1977; Campbell and Scanes 1985).

The implication of GH in lipid metabolism prompted us to search for DNA polymorphisms in the GH gene and to test whether the incidence of such polymorphisms is affected by selection for fatness and leanness, respectively. Analysis of two strains derived from the same genetic base, but selected divergently for abdominal fat content, revealed the presence of several DNA polymorphisms in the GH gene. The incidence of two of these polymorphisms differed between the two lines at a high level of significance. Although correlation studies between GH expression

Correspondence to: U. Kuhnlein

and genotype have not yet been conducted, this result indicates that allelic variations in the GH gene may influence fatness and leanness.

Materials and methods

Strains

The derivation of the fat line (FL) and lean line (LL) and their properties have been described in detail by Leclerq (1988). Briefly, a base population was established from chickens of six different meat-type strains of widely different origin. Selection for leanness and fatness was on the sire side and was based on the weight of the abdominal fat pad. In each generation between nine and 15 sires and 14 to 67 dams were used as breeders. After selection for six generations, the two strains were imported to our poultry unit in 1982 and maintained non-selected. Between 60 and 70 sires and the same number of dams were used to propagate each strain. The average amount of abdominal fat per live weight in 1982 was 37.1 ± 12.4 g/kg in the fat line and 12.3 ± 5.6 g/kg in the lean line (Leclerq 1988).

Identification of GH DNA polymorphisms

DNA was isolated from 100 µl of heparinized blood according to Jeffreys and Morton (1987) and dissolved in 5 mM Tris-HCl, 0.1 mM EDTA (pH 7.5). An aliquot of 5 µg of DNA was digested with 25 units of restriction enzyme overnight and subjected to electrophoresis in a 1% agarose gel for 12 h at 1 V/cm. After blotting onto Zeta probe membrane (Bio-Rad), the GH fragments were visualized by hybridisation to a ^{32}P-labelled full-length turkey growth hormone DNA probe (Karatzas et al. unpublished) followed by autoradiography. Prehybridisation, hybridisation and washing were performed according to Kuhnlein et al. (1989).

DNA fingerprinting

DNA fingerprinting using $(CAC)_5$ as a probe was carried out according to Ali et al. (1986). Five µg of genomic DNA from seven chickens of the fat line and seven chickens of the lean line were digested overnight at 37 °C with 25 units of *Alu*I. Electrophoresis was carried out for 21 h at 1 V/cm in an 0.7% agarose gel in 1 x TPE buffer (Sambrook et al. 1989). The gel was dried in a vacuum-gel drier for 30 min at room temperature followed by 30 min at 60 °C. The gel was then soaked, for 30 min each, in 0.5 M NaOH/0.15 M NaCl and 0.5 M Tris/0.15 M NaCl (pH 8.0) followed by equilibration with 6 x SSC. Prehybridisation was carried out at 43 °C for 15 min in 5 x SSPE (0.9 M NaCl, 5 mM NaH_2PO_4, 1 mM EDTA, pH 7.4), 5 x Denhardt's solution (Sambrook et al. 1989) and 10 µg/ml of denatured herring sperm DNA. Hybridisation with ^{32}P-labelled $(CAC)_5$ was carried out in the same solution at 43 °C for 2–3 h. The probe was end-labelled with (γ^{32}P)-ATP and T4 polynucleotide kinase. After hybridisation, the gel was washed in 6 x SSC for 3 x 30 min at RT followed by a 1-min wash at 43 °C. The gel was blotted dry and exposed to an X-ray film for 2 days at RT.

The average band-sharing coefficients were determined as outlined by Kuhnlein et al. (1990). Briefly, the eight most-intense bands in each of the individuals to be compared were marked. Each individual was then tested for the presence or absence of these bands, taking into account that the maximum difference of intensity could not exceed a factor of two (homozygote vs heterozygote). The band-sharing between individuals A and B was then computed as $2N_{AB}/(N_A + N_B)$, where N_{AB} was the number of bands shared and N_A and N_B the number of bands scored in individuals A and B, respectively. These individual

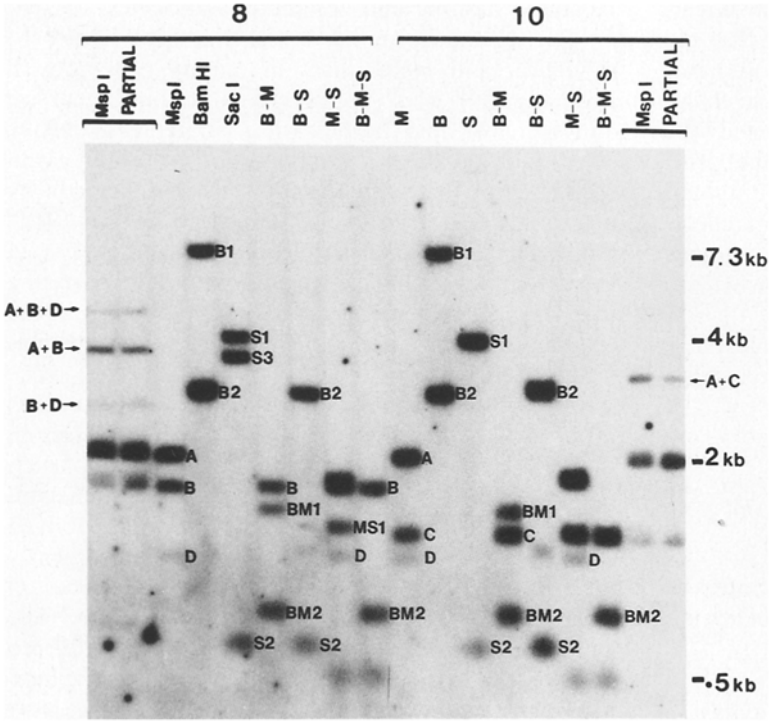

Fig. 1. Restriction enzyme analysis of two chickens of the fat line. Single-, double- and triple-digests with the enzymes *Msp*I, *Bam*HI and *Sac*I, as well as partial digests with *Msp*I, were analyzed. Chicken #8 was homozygous at *Msp*I sites, yielding fragments *A*, *B* and *D*, and heterozygous at a *Sac*I site, yielding the two bands *S1* and *S3*. Chicken #10 was also homozygous at *Msp*I sites, yielding fragments *A*, *C* and *D*, and homozygous at a *Sac*I sites. Restriction enzymes and conditions are marked on top of the figure and fragments can be identified by comparison with Fig. 3

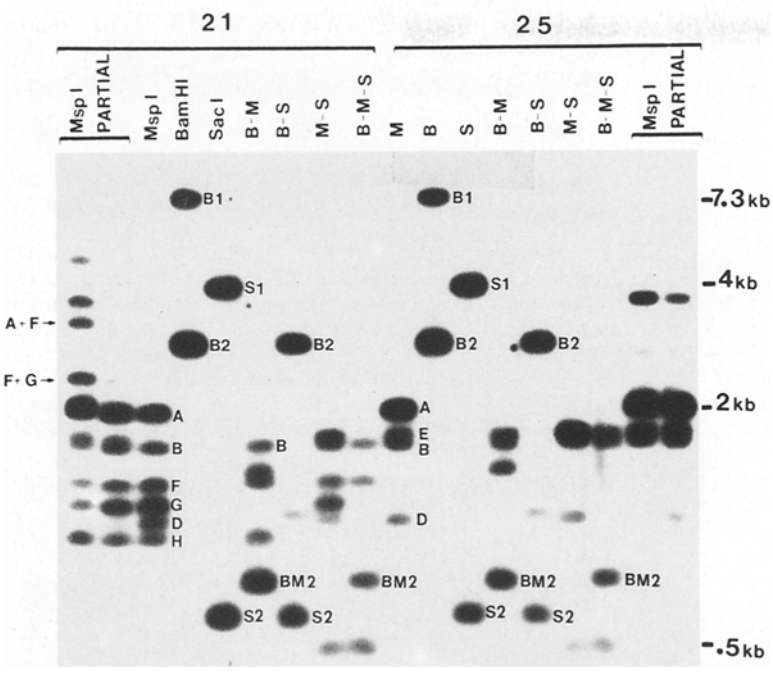

Fig. 2. Restriction enzyme analysis of two chickens of the lean line. Single-, double- and triple-digests with enzymes *Msp*I, *Bam*HI and *Sac*I, as well as partial digests with *Msp*I, were analyzed. Chicken #21 was heterozygous at three *Msp*I restriction sites (PM1, PM2 and PM3) giving rise to bands *A*, *B*, *F*, *G*, *D*, and *H*. Chicken #25 was heterozygous at the *Msp*I restriction site PM2, giving rise to bands *E*, *B* and *D*. Restriction enzymes and conditions are marked on top of the figure and fragments can be identified by comparison with Fig. 3

band-sharing coefficients were then averaged over all possible pair-wise combinations.

Mapping of the GH polymorphisms in chickens of the fat and lean lines

A gene map was established by analyzing partial *Msp*I digests, as well as single-, double- and triple-digests, with *Bam*HI, *Sac*I and *Msp*I. The probes used in these analyses were the entire turkey c-DNA (894 base pairs) and two *Msp*I subfragments (nucleotides 0–370 and 370–800). They will be referred to as the 5′-probe and the 3′-probe, respectively. The autoradiographs from which the map was derived are shown in Figs. 1 and 2. All the fragments whose arrangement is discussed below are indicated in Fig. 3.

Chicken #8 of the fat line was analyzed first (Fig. 1). It was homozygous at *Msp*I sites, yielding the fragments A (2.0 kb), B (1.6 kb) and D (1.0 kb). Fragment A hybridized with the 3′-probe but not the 5′-probe and, therefore, had to be located on the 3′-side of B and D (data not shown). Partial digests with *Msp*I yielded three additional bands of molecular weight (MW) 2.6 kb, 3.6 kb and 4.8 kb, respectively. These molecular weights were consistent with the precursors B + D, A + B and A + B + D. Hence, the order of the fragments in the 5′ to 3′ direction had to be D, B, A. This order was supported by the observation that the 5′-probe hybridized preferentially to the precursor B + D, while the 3′-probe hybridized preferentially to the precursor A + B (data not shown). Partial digests also revealed two additional bands about 0.1 kb above band A and band B, which indicated the presence of two *Msp*I sites at the boundary of A and B, spaced about 0.1 kb apart.

Chicken #10 of the fat line was also homozygous at *Msp*I sites, but differed from chicken #8 by one *Msp*I polymorphism (Fig. 1). Instead of band B (1.6 kb), band C was observed which had a MW of 1.2 kb. Partial digests yielded two additional bands of MW 3.2 kb and 1.6 kb (weak). The 3.2 kb band suggests that C was adjacent to A and the 1.6 kb band suggests that B was a precursor of C. In addition, partial digests revealed bands which are 0.1 kb larger than A and C, respectively, again indicating the presence of two *Msp*I sites at the boundary of A and C. The additivity of the molecular weights of the *Msp*I fragments indicated that there were no large *Msp*I fragments (> 0.1 kb) which lay entirely in introns. Thus, the arrangement of fragments A, B, C and D had to be as shown in Fig. 3.

Digestion with *Bam*HI yields two fragments, B1 (7.3 kb) and B2 (2.9 kb), in both chickens (Fig. 1). The 7.3 kb bands did not hybridize with the 5′-probe and thus had to be 3′ of B2.

*Bam*HI/*Msp*I double-digests showed that fragments A and D, but not B or C, had *Bam*HI sites. The two new fragments, BM1 (1.4 kb) and BM2 (0.7 kb), presumably originated from fragment A, since their molecular weights added up to the MW of fragment A and, like A, they did not hybridize with the 5′-probe.

*Sac*I digestion of DNA from chicken #10 yielded the two fragments S1 (4.0 kb) and S2 (0.6 kb). S2 hybridized only with the 3′-probe and thus had to be 3′ of S1. *Bam*HI/*Sac*I double-digests indicated that B2 did not contain a *Sac*I site. Hence, S1 had to comprise the entire B2 fragment and S2 was nested within B1.

*Sac*I/*Msp*I double-digests show that none of the *Msp*I fragments contained *Sac*I sites with the exception of fragment A. As indicated above, fragment A also had a *Bam*HI site. Triple-digests with *Bam*HI, *Sac*I and *Msp*I indicated that this *Sac*I site was located in BM1.

Chicken #8 was heterozygous for a *Sac*I polymorphism, resulting in an additional *Sac*I fragment (S3) of 3.5 kb. This polymorphism did not affect the restriction patterns in double- and triple-digests with the exception of the double-digest *Msp*I/*Sac*I, where an additional fragment MS1 (1.3 kb) was observed. This band hybridized only with the 3′-probe and thus had to arise from an additional *Sac*I site 0.5 kb from the 3′-end of S1.

Among the chickens of the lean line three additional *Msp*I polymorphisms were observed (Fig. 2). One of these stemmed from an additional *Msp*I restriction site in fragment A (2.0 kb), yielding two fragments G (1.1 kb) and H (0.9 kb). These two fragments did not hybridize with the 5′-probe, as expected if

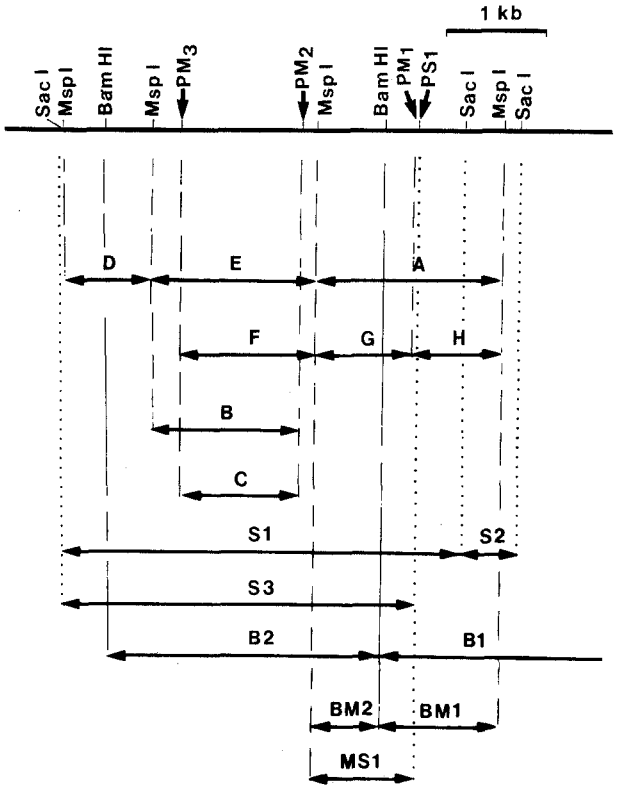

Fig. 3. Restriction map of the growth hormone gene. The fragments relevant to its derivation are indicated

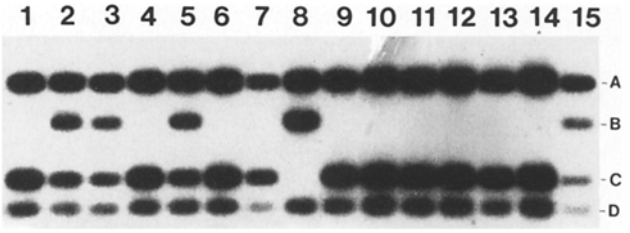

Fig. 4. *Msp*I polymorphisms in 15 chickens of the fat line. The polymorphic *Msp*I sites which give rise to the observed banding patterns are indicated in Fig. 3

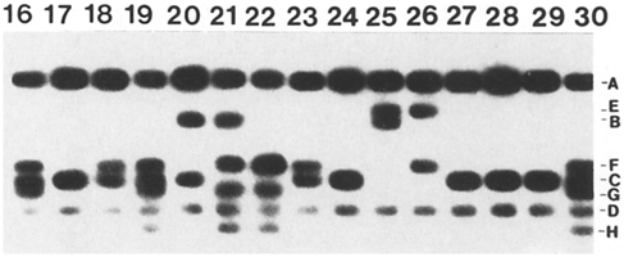

Fig. 5. *Msp*I polymorphisms in 15 chickens of the lean line. The polymorphic *Msp*I sites which give rise to the observed banding patterns are indicated in Fig. 3

they originated from fragment A. Double-digests indicated that G had a *BamHI* site and H had a *SacI* site. Thus G had to be 5′ of H. Chicken #21 was heterozygous for this polymorphism.

A second polymorphism in the lean line was observed at one of the *Msp*I sites flanking fragment B. Chicken #25 was heterozygous for B (1.6 kb) and E (1.8 kb). Since fragments of 2.1 kb and 1.7 kb were seen in partial digests of chickens homozygous for A (2.0 kb) and B (1.6 kb), we presume that E arose from a polymorphism at one of the closely spaced *Msp*I sites at the boundary between A and B. Partial digests indeed showed a strongly hybridizing band at 3.8 kb.

A third *Msp*I polymorphism which also affected fragment B was analyzed in chicken #21 (Fig. 2). It resulted in a MW change of fragment B (1.6 kb) to fragment F (1.3 kb). Partial digests yielded a strong band at MW 3.3 kb which indicated that fragment F was adjacent to fragment A (2.0 kb). In addition, a partial digest yielded a second intense band at 2.3 kb which presumably represented the precursor of G (1.1 kb) and F (1.3 kb). This precursor was expected if G was indeed 5′ of H.

Results and discussion

Analysis of *Msp*I digests of the genomic DNA of chickens from the fat and lean lines revealed that the growth hormone gene is polymorphic. In chickens from the fat line a single *Msp*I polymorphism was observed, resulting in a shift of the molecular weight of one band (Fig. 4). In chickens from the lean line multiple polymorphisms were observed (Fig. 5). One of these gave rise to a band of MW 2.0 kb or two bands of MW 1.1 kb and MW 0.9 kb, respectively. Two additional polymorphisms affected the MW of a single band.

Analysis of *Sac*I digests revealed the presence of a fourth polymorphism which was present in the fat line but not in the lean line. It resulted in a MW shift of a single band from 4.0 to 3.5 kb (Figs. 1, 3).

The individual genotypes were first derived by comparing band densities (homozygotes vs heterozygotes) and band associations, and then confirmed by restriction mapping of some selected individuals. The construction of a restriction map of the chicken GH gene and the location of the GH polymorphisms was based on the analysis of single-, double- and triple-digests with *Bam*HI, *Sac*I and *Msp*I, as well as partial digests with *Msp*I (see Materials and methods and Fig. 3). This map indicated that all major hybridizing sequences of the growth hormone cDNA were located within a genomic DNA fragment of 4.0 kb and that all four polymorphisms were located in introns and/or exons, but not in flanking regions of the gene.

The frequencies of the four polymorphisms are shown in Table 1. Polymorphisms at the PS1 restriction site were rare and were only observed in the fat line. Segregation for a polymorphism at the PM3 restriction site was more frequent, but did not

Table 1. Incidence of GH polymorphisms in the fat and lean lines

Site[a]	Restriction enzyme	Incidence of polymorphisms[b]	
		Fat line (N = 40)	Lean line (N = 40)
PS1	*Sac*I	4	0
PM1	*Msp*I	0	7[c]
PM2	*Msp*I	40	24[c]
PM3	*Msp*I	31	34

[a] The location of the sites is shown in Fig. 3
[b] Number of alleles where the restriction site is present
[c] Lines differ significantly in the frequency of polymorphisms at PM2 ($P < 0.004$) and PM1 ($P < 0.02$) by Fisher's exact probability, two-tailed, test

Table 2. Incidence of GH genotypes in the fat and lean lines

Genotype[a]				Fat line (N = 20)	Lean line (N = 20)
PS1	PM1	PM2	PM3		
+/−	−/−	+/+	+/−[b]	3	0
+/−	−/−	+/+	−/−	1	0
−/−	−/−	+/+	+/+	12	5
−/−	−/−	+/+	+/−	4	1
−/−	−/−	+/−	+/−	0	1
−/−	+/−	+/−	+/+[b]	0	5
−/−	+/−	+/−	+/−[b]	0	1
−/−	+/−	−/−	+/+	0	1
−/−	−/−	+/−	+/+	0	3
−/−	−/−	+/−	−/−	0	2
−/−	−/−	−/−	+/−	0	1

[a] The absence of a restriction site is indicated by − and the presence by +. The locations of the restriction sites within the GH gene are shown in Fig. 3
[b] Genotypes with two possibilities of assigning alleles. Note that all four possible combinations of the polymorphisms at PM2 and PM3 can be distinguished on the Southern blot

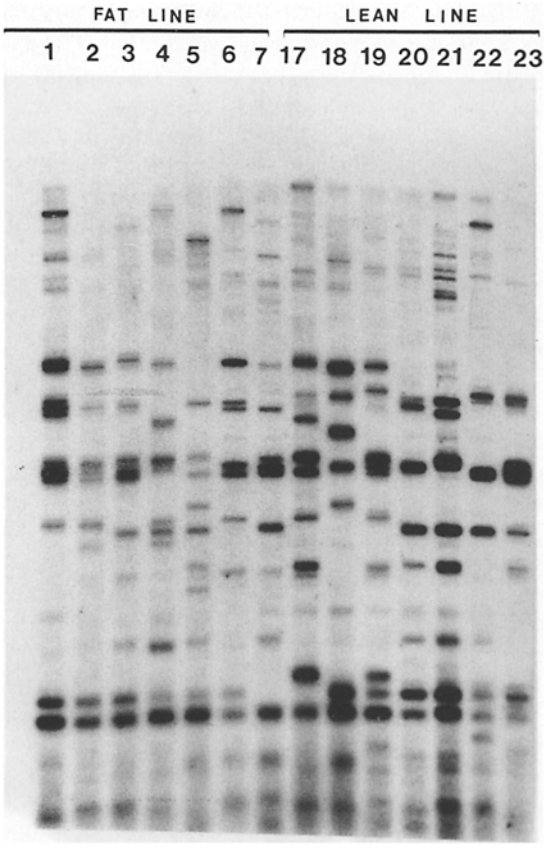

Fig. 6. DNA fingerprint of seven fat and lean line chickens using CAC_5 as a probe

Table 3. Average band-sharing coefficients within and between lines

Line	Number of comparisons[a]	Band-sharing[b] (mean ± SD)
Fat	21	0.50 ± 0.14
Lean	21	0.51 ± 0.12
Fat/lean	49	0.45 ± 0.11

[a] In each line seven individuals were analyzed, giving rise to 21 pair-wise comparisons within each line and 49 comparisons between lines
[b] The band-sharing coefficients did not differ significantly from each other (t-test, $P > 0.05$), indicating the two strains had the same inbreeding coefficient and that there was no significant genetic drift

differ significantly between the two strains. The frequencies of polymorphisms at PM1 and PM2, however, were significantly different. The polymorphisms at PM1 and PM2 were fixed in the fat line but segregated in the lean line.

The four polymorphisms give rise potentially to four alleles in the fat line and eight alleles in the lean line. The number of alleles deduced from the observed genotypes (Table 2) were 3–4 for the fat line and 5–7 for the lean line (the ambiguity in this determination stems from heterozygosity at several restriction sites in the same individual). The alleles which were not observed were expected to occur at low frequencies (< 0.1). The results indicate that the GH gene in chickens is highly polymorphic and that nearly all of the alleles representing different permutations of polymorphisms are present. This may reflect the extremely broad range of breeds used to establish the base population (Leclercq 1988).

The lower number of alleles present in the fat line indicates that selection for fatness may have provided a stronger selection pressure on the GH alleles than selection for leanness. However, most alleles occurred at frequencies below 0.2, with the exception of the

allele $PS1^{-}PM1^{-}PM2^{+}PM3^{+}$ which, based on the frequency of homozygotes and assuming Hardy-Weinberg equilibrium, was present at frequencies of 0.50 and 0.77 in the lean and fat line, respectively.

In order to ensure that differences in the incidence of the GH polymorphisms was not due to random genetic drift associated with inbreeding, the two strains were analyzed by DNA fingerprinting (Fig. 6). The average band-sharing coefficients of individuals within each of the two strains were similar (Table 3) and corresponded to an inbreeding coefficient of 0.1 (Kuhnlein et al. 1990). Further, the two intra-strain band-sharing coefficients were not significantly different from the inter-strain band-sharing coefficient obtained by comparing chickens belonging to the fat and lean line, respectively (Table 3). Thus, DNA fingerprinting did not indicate extensive random genetic drift.

In summary, we have shown that the GH gene in chickens is highly polymorphic. Selection for leanness and fatness, respectively, had a differential influence on the incidence of a polymorphism at two *Msp*I restriction sites which are located in the central area of the GH gene. Whether this response reflects an altered expression or structure of GH associated with the particular alleles or whether it is due to linkage to a neighbouring gene requires further analysis.

Acknowledgements. The authors thank Ms. L. Volkov for her assistance with blood collection and genomic DNA isolation. The research was supported by a grant from the National Sciences and Engineering Research Council of Canada and by Shaver Poultry Breeding Farms Ltd.

References

Ali S, Muller CR, Epplen JT (1986) DNA fingerprinting by oligonucleotide probes specific for simple repeats. Human Genet 74:239–243

Breier BH, Gluckman PB, McCutcheon SN, Davis SR (1989) Physiological responses to somatotropin in the ruminant. J Dairy Sci 74:20–34

Campbell RM, Scanes CG (1985) Lipolytic activity of purified pituitary and bacterially derived growth hormone on chicken adipose tissue in vitro. Proc Soc Exp Biol Med 180:513–516

Harvey S, Scanes CG, Howe T (1977) Growth hormone effects on in-vitro adipose and liver tissue. Gen Comp Endocrinol 33:322–328

Jeffreys AJ, Morton DB (1987) DNA fingerprints of dogs and cats. Anim Genet 18:1–15

Kuhnlein U, Gavora JS, Spencer JL, Bernon DE, Sabour M (1989) Incidence of endogenous viral genes in two strains of White Leghorn chickens selected for egg production and susceptibility or resistance to Marek's disease. Theor Appl Genet 77:36–32

Kuhnlein U, Zadworny D, Dawe Y, Fairful RW, Gavora JS (1990) Assessment of inbreeding by DNA fingerprinting: development of a calibration curve using defined strains of chickens. Genetics 125:161–165

Leclercq B (1988) Genetic selection of meat-type chickens for low abdominal fat content. In: Leclercq B, Whitehead CC (eds) Leanness in domestic birds. Butterworth, London Boston Singapore Sydney Toronto Wellington, pp 25–40

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory handbook. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Scanes CG (1987) The physiology of growth, growth hormones and other growth factors in poultry. CRC Crit Rev Poultry Biol 1:51–105

Whitehead CC (1988) Selection for leanness in broilers using plasma lipoprotein concentration as a selection criterion. In: Leclercq B, Whitehead CC (eds) Leanness in domestic birds. Butterworth, London Boston Singapore Sydney Toronto Wellington, pp 41–57