Evidence for a Genetic Variation in the Mitochondrial Genome Affecting Traits in White Leghorn Chickens

S. Li, S. E. Aggrey, D. Zadworny, W. Fairfull, and U. Kuhnlein

A mitochondrial *Msp*I RFLP which was coselected with Marek's disease (MD) resistance in White Leghorn chickens was mapped to the NADH subunit IV. The RFLP was due to a transition, resulting in the change of the low-usage threonine triplet ACT (*Msp*I$^-$ allele) to the high usage triplet ACC (*Msp*I$^+$ allele). Trait association studies within an unselected strain revealed that the *Msp*I$^-$ allele whose frequency was reduced in MD resistant strains was associated with high body weight and high egg specific gravity (a measure of eggshell thickness). Analysis at three different time points indicated a significant interaction between the mitochondrial genotype and the growth hormone genotype in early but not in late adulthood. The analysis indicates that mitochondrial variants may contribute to phenotypic variation in chickens and that such contributions may be dependent on the genetic background.

Mitochondria play a central role in energy metabolism, amino acid metabolism, fat metabolism, and steroidogenesis (Whittaker and Danks 1978). Although being an autonomously replicating organelle, most of the proteins present in the mitochondrion are encoded by nuclear genes (Anderson et al. 1981). The genes still present in the mitochondrial genome are coding for mitochondrial tRNA, rRNA, and for genes coding for some of the subunits of the protein complexes responsible for oxidative phosphorylation (OXPHOS) complexes. The mitochondrial genes are devoid of regulatory elements and tissue-specific regulation of mitochondrial genesis and metabolic activity is mediated by cellular genes. Nevertheless, in humans many mutations in mitochondrial genes have been found to affect mitochondrial function. OXPHOS diseases include missense mutations, tRNA mutations, and insertion-deletion mutations. Such mutations affect mainly organs which are dependent on mitochondrial energy, such as the nervous system, muscle, heart, pancreatic islands, kidney, and liver. Further, symptoms in these diseases are mostly progressive, presumably because reduced mitochondrial activity is compounded by the loss of functional mitochondria as part of the aging process (Wallace 1992).

Inheritance of mitochondria is predominantly maternal, presumably because the number of mitochondria present in the ovum exceeds the number of mitochondria carried by the sperm by several orders of magnitude (Gyllenstein et al. 1991). An additional unique feature of mitochondrial DNA is the lack of recombination and the high frequency of mutations, which is about 10 to 20 times higher than for the nuclear DNA (Merriwhether et al. 1991). The potential number of mutations which affect traits may therefore be quite high.

In a screen of an anonymous chicken liver cDNA library for clones which revealed restriction length polymorphisms (RFLP) at *Msp*I sites in White Leghorns, we found that several clones were of mitochondrial origin and that the associated RFLP was coselected with egg production and/or disease resistance. All mitochondrial clones revealed the same *Msp*I RFLP (unpublished results). In this communication we identified the molecular nature of this RFLP and tested whether it was associated with body weight and egg quality traits. We further investigated the interaction between the mitochondrial genotype and a marker in the growth hormone gene, a gene associated with body weight as well as with egg production traits (Kuhnlein et al. 1997; Kuhnlein and Zadworny 1994).

Materials and Methods

Strains of Chickens and Measurement of Traits

Strain 7 is a White Leghorn strain established in 1958 by cross-mating 4 North

From the Department of Animal Science, McGill University, Ste. Anne de Bellevue, Quebec, Canada H9X 3V9 (Li, Aggrey, Zadworny, and Kuhnlein) and the Centre for Food and Animal Research, Agriculture Canada, Ottawa, Ontario, Canada (Fairfull). This study was supported by the Natural Sciences and Engineering Research Council of Canada and Shaver Poultry Farms Ltd. We wish to thank the Centre for Food and Animal at Agriculture Canada, Ottawa, for providing us with DNA samples and production traits. Special thanks to L. Asselstine and L. Volkov for their help in poultry management, trait measurement, and genotyping. Address correspondence to Dr. Kuhnlein at the address above or e-mail: kuhnleinu@agradm.lan.mcgill.ca.

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Table 1. Frequency of the mitochondrial *MspI*$^+$ allele in White Leghorn strains of different genetic origin

Genetic base	Strain[a]	Selection criteria	MD-resistance	Frequency of the *MspI*$^+$ allele
I	7	Control strain	Low	0.30
	8	Selected for egg production traits	Intermediate	0.42
	9	Selected for MD resistance	Unknown	0.41
	8R	Selected for MD resistance	High	0.56
II	3	Selected for egg production traits	Intermediate	0.00
	3R	Selected for MD resistance	High	0.00
III	S	Selected for MD susceptibility	Low	0.00
	K	Selected for MD resistance	High	0.00
	Rs	Selected for ALV usceptibility	Low	0.00
	Rr	Selected for ALV resistance	High	0.00
IV	Gs	Selected for ALV susceptibility	Low	0.68
	Gr	Selected for ALV resistance	High	0.83
V	Mr	Selected for ALV resistance	Low	0.43
	Ms	Selected for ALV susceptibility	High	0.90

[a] Strains 7, 8, 9, 8R, 3, and 3R have been described (Gavora et al. 1989; Gowe et al. 1993). Strains S and K were developed at Cornell and were selected for MD resistance (Cole and Hutt 1973). Strain Rs and Rr were derived from a substrain of strain K under divergent selection for tumor formation in response to wing injection with Rous sarcoma virus (Hartmann et al. 1984). Strains Gs and Gr and Ms and Mr were selected as Rr and Rs, but were of North American and German origin, respectively. MD mortality in Gr and Ms was greater than in Gs and Mr, respectively (Hartmann et al. 1984). Sample sizes were 20, with the exception for strains of genetic base IV and V where 30 individuals were typed.

American commercial strains and was propagated by random mating at an effective population size of 457 without selection. The strain analyzed here is the generation raised in 1993. Strains 8 and 9 were derived from strain 7 in 1969 and propagated under selection for an array of egg production traits at an effective population size of 184 (Gowe et al. 1993). All other strains analyzed in this study are described in Table 1.

Body weights were measured at 130, 265, and 365 days of age. Egg weights and the egg specific gravities were measured on up to 5 eggs collected from each hen starting from 240, 350, and 450 days of age, respectively. Egg specific gravity, a measure of eggshell thickness, was measured by submerging eggs in NaCl solutions of increasing density.

Southern Blotting

DNA was extracted from 60 μl of heparinized whole blood as described by Jeffreys and Morton (1987) and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 7.5). Five micrograms of DNA was digested with *Msp*I or *Taq*I restriction enzyme and subjected to electrophoresis on a 1% agarose gel at 1.25 V/cm for 20 h. The DNA was transferred to a nylon membrane (Bio-Rad) by alkaline blotting (Reed and Mann 1985). Probes were labeled by random primer extension (^{T7}Quick-Prime Kit; Pharmacia) and hybridization was carried out as described previously (Kuhnlein et al. 1989).

Polymerase Chain Reaction

The primer sequences used to amplify the mitochondrial segment containing the *Msp*I RFLP locus were 5′-CAGGCCTAGCCATACAAGTAG-3′ (forward primer) and 5′-TAAGCTTGTTCAGGAGGCAGG-3′ (reverse primer). The reactions (25 μl) contained 2 units of *Tth* polymerase, 0.2 μg of DNA, 20 μM of each primer, 125 μM of each nucleotide, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 20 mM $MgCl_2$, and 0.01% gelatin. After denaturing for 10 min at 95°C, a total of 40 cycles were carried out, each consisting of 1 min at 94°C, 80 s at 59°C, and 90 s at 72°C. The PCR products were digested with *Msp*I enzyme and analyzed by electrophoresis in a 1.5% agarose gel. The PCR-RFLP assay for the *Sac*I RFLP in intron 4 of the chicken growth hormone has been described (Kuhnlein et al. 1997).

Direct Sequencing of PCR Products

Twenty-five microliters of PCR product was purified by electrophoresis in a 1.5% low-melting agarose gel containing ethidium bromide in 1× TAE buffer. The band containing the DNA was cut out, placed in 1 volume of distilled water, and heated at 95°C for 5 min. Five microliters of the preparation was used for direct sequencing using the "dsDNA cycle sequencing system" and following the instructions of the supplier (GIBCOBRL).

Statistical Analysis

The association of the two mitochondrial marker genotypes (haploid) with traits was analyzed by single-factor analysis of variance. The combined association mitochondrial and GH genotypes were analyzed by two-factor analysis of variance (type III) using the model $y_{ijk} = mitgeno_i + ghgeno_j + mitgen_i \times ghgeno_j + e_{ijk}$. The deviation of the observed from the expected genotype frequencies, that is, Hardy-Weinberg equilibrium for the GH genotypes and random association with the mitochondrial genotype, were assessed by chi-square analysis df = 4.

The discussion on genetic drift versus selection is based on the following argument. In the case of extreme random genetic drift, an allele may become fixed in some subpopulations, whereas in the remaining subpopulations allele frequencies will have an equal probability to take any value between 0 and 1 (Kimura 1955). Assuming that in the segregating subpopulations the allele frequencies are μ_1 and μ_2, respectively, and $\mu_1 < \mu_2$, then the probability to obtain a more extreme outcome than observed is $\frac{1}{2}(1 + \mu_1 - \mu_2)^2$ multiplied by a factor two to account for the random order of allele designation. If several divergently selected pairs are analyzed, the overall probabilities multiply, but the correction factor of two only applies once, since the order of alleles becomes fixed in the first comparison. The mathematical treatment in the case of moderate random drift is complex. However, inspection of the allele frequency distributions given by Kimura (1955) indicates that the probability of observing a given allele frequency between subpopulations is even lower (cf. Kuhnlein et al. 1997).

Results

Identification of the Mitochondrial RFLP

Bulk screening of a liver cDNA library for clones which revealed *Msp*I or *Taq*I RFLPs in a series of White Leghorn strains led to the identification of four clones with sequence homology to the ATPase-6 gene and the tRNA-serCOII region (Desjardins and Morais 1990). Based on the coincidence of RFLP segregation among individuals and restriction fragment size, all four clones revealed the same *Msp*I RFLP, located in the flanking region of the clone. No RFLPs were found at *Taq*I restriction sites.

Southern blotting indicated that the RFLP produced a single band shift from 4700 bp to 4100 bp (Figure 1). A fragment of 4700 bp was expected from the location

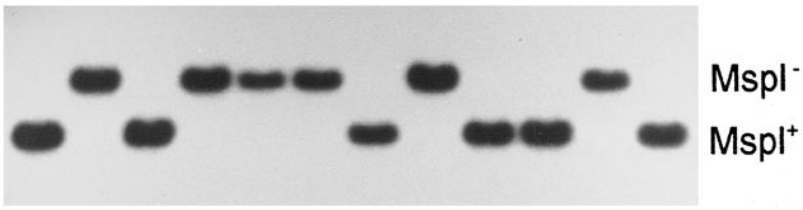

Figure 1. Southern blot of *Msp*I-digested DNA from chickens of strain 7 hybridized with the mitochondrial clone CLEST 20. The two alleles *Msp*I$^-$ (absence of an *Msp*I restriction site) and *Msp*I$^+$ (presence of an *Msp*I restriction site) are indicated.

of two *Msp*I sites located at position 8901 and 12,673, respectively, indicating that the *Msp*I$^+$ allele (presence of an *Msp*I site) originated from an additional *Msp*I site not present in the published sequence (Desjardin and Morais 1990). Double digests with other restriction enzyme placed this *Msp*I site close into the 3 end of this fragment. Appropriate PCR primers were designed and the location of the RFLP was confirmed by restriction analysis of the amplified DNA fragment.

The identity of the base change was determined by direct sequencing of the PCR product obtained from the DNA of two individuals with different RFLP alleles. Compared to the analysis of subcloned PCR products, direct sequencing is expected to lower the frequency of errors which may occur during amplification. A nucleotide change from T to C was found at position 11,998, resulting in the creation of an additional *Msp*I site (Figure 2). This nucleotide substitution was in the coding region for NADH dehydrogenase subunit IV, but did not alter the amino acid sequence. No other changes between the two RFLP alleles was found in 300 bases in the vicinity of the *Msp*I site.

Homoplasy and Incidence of the *Msp*I RFLP in White Leghorn Strains

DNA was isolated from red blood cells, which contain an average of 8 mitochondria. Within the limits of resolution of the PCR assay (admixture of the two genotypes $\geq$ 1:9) nearly all chickens segregated for a single mitochondrial genotype, as expected from maternal inheritance of mitochondria. Heteroplasy, the presence of both mitochondrial alleles, was only observed once among more than 400 individuals tested. Whether heteroplasy in this individual was due to a mixture of the two genotypes in single red blood cells or reflected an admixture of red blood cells of unique genotype was not determined.

Analysis of the RFLP in White Leghorn strains originating from different genetic origins revealed segregation in three of the five sets of strains (Table 1). Set one consisted of strains 7, 8, 9, and 8R which had been established from four different North American commercial strains in 1956 (Gowe et al. 1993); set two comprised strains Mr and Ms which were derived from a North American commercial strain; and set three comprised strains Gr and Gs which had been derived from a strain of German origin. The latter two strains had been divergently selected for the susceptibility to tumor formation upon wing web injection of Rous sarcoma tumor virus (Hartmann et al. 1984). Two other sets of strains, one of Canadian origin and one developed at Cornell in the late 1930s, were fixed for the *Msp*I$^-$ allele. Marek's disease (MD) resistant strains had an increased incidence of the *Msp*I$^+$ allele as compared to their more susceptible related strains (8 versus 8R, Gs versus Gr, and Mr versus Ms). As outlined in the Materials and Methods section, the consistent differences are unlikely to be the result of random genetic drift ($P \leq .03$). However, in the divergently selected strains G and M, selection for MD resistance was not part of the selection criteria and the origin of the hidden selection which may have led to differences in MD resistance is unknown. Nevertheless, a maternally inherited component of MD resistance has been reported (Hartmann et al. 1991).

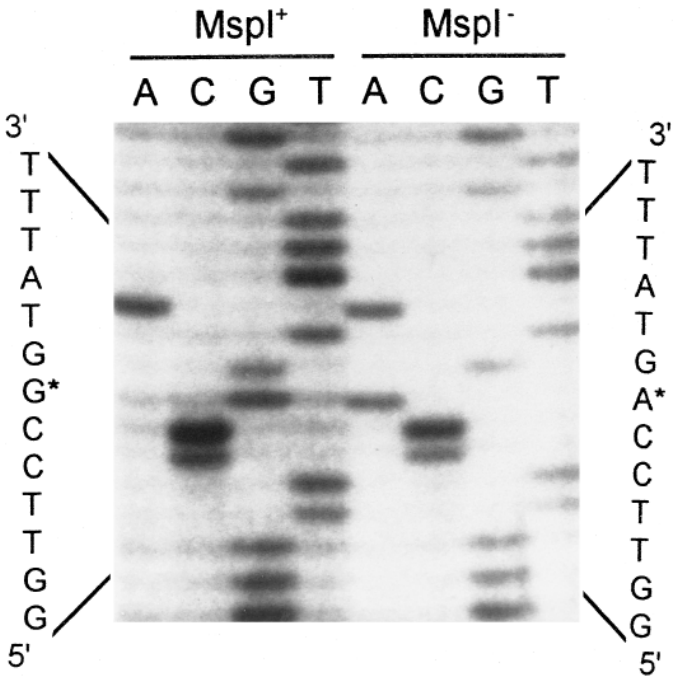

Figure 2. Direct sequencing of the PCR product of the polymorphic region of an *Msp*I$^+$ and an *Msp*I$^-$ allele from strain 7. The sequence corresponds to the noncoding strand.

Trait Association in a Nonselected White Leghorn Population

Comparison of the control strain (strain 7) with two substrains selected for a wide array of egg production related traits (strains 8 and 9) indicated coselection of the *Msp*I$^+$ allele (Table 1). Further evidence from typing extreme phenotypes for a series of traits (data not shown) prompted us to analyze trait association of the mitochondrial genotype in more detail.

The association study was carried out in strain 7, a strain of White Leghorns which had been kept nonselected since 1956 at an average effective population size of 457 and segregated for the mitochondrial *Msp*I$^+$ allele at a frequency of 0.30. Analysis of variance was carried out for body weight, egg weight, and egg specific gravity at three different ages (Table 2). Egg specific gravity is highly correlated with eggshell thickness.

Significant associations were found for two of the three traits, body weight and specific gravity. For body weight the association was significant at 365 days of age

Table 2. Least-square means of body weight, egg weight, and egg specific gravity in dependence of the mitochondrial genotype in strain 7

Trait	Coefficient of variation	Mitochondrial genotype		Significance ($P > F$)
		*Msp*I$^+$ (N = 104)	*Msp*I$^-$ (N = 237)	
Body weight (g)				
130 days	10.4	1,276	1,302	0.107
265 days	12.0	1,718	1,763	0.073
365 days	13.3	1,740	1,802	0.028
Egg weight (g)				
240 days	6.70	52.9	52.5	0.310
350 days	7.36	58.5	58.1	0.371
450 days	7.78	60.6	60.7	0.843
Egg specific gravity[a]				
240 days	5.40	84.7	85.8	0.063
350 days	5.65	81.3	82.1	0.181
450 days	6.86	78.3	79.5	0.095

[a] The units for egg specific gravity are -1×10^4 g.

(mature body weight), where the mean for the *Msp*I$^+$ genotype was 66 g lower than for the *Msp*I$^-$ genotype. The same trend but of a lesser magnitude was observed at earlier ages. Egg specific gravity was also reduced in chickens with an *Msp*I$^+$ mitochondrial genotype, and this difference was significant at 240 days of age.

Both body weight and egg specific gravity are complex multigenic traits and expected to be influenced by allelic variations in many different genes. In strain 7 we had previously characterized alleles of the GH gene, another candidate gene for growth and production related traits. Among the three alleles segregating in strain 7, the major allele A1 was increased in substrains selected for egg production and related traits and could be distinguished from the other alleles by virtue of a *Sac*I RFLP in intron 4 (Kuhnlein et al. 1997; Kuhnlein and Zadworny 1994).

The combined effect of the marker genotypes at the two loci is shown in Tables 3 and 4. For body weight at 130 days, the interaction between the mitochondrial and GH genotypes was significant ($P \leq .034$). Analysis of individual genotypic classes revealed that the interaction was due to a large effect of the mitochondrial genotype in conjunction with the A1$^-$/A1$^-$ GH genotype, while effects in the other GH genotype classes, although in the same direction, were small. At 265 days of age, none of the genotypes had a significant effect, while at 365 days of age the effect of the mitochondrial genotype was again significant, but not restricted to a particular GH genotypic class.

A similar effect was observed for egg specific gravity. At an early age, the effect of the mitochondrial genotype was only significant in the GH genotype classes A1$^-$/A1$^-$ and A1$^+$/A1$^-$. At 350 days of age there was no significant effect of the mitochondrial genotype at all, while at 450 days of age the effect of the mitochondrial genotype was significant, but independent of the GH genotype.

Table 3. Significance of the mitochondrial and GH genotype and their interaction in strain 7

Trait	Significance ($P > F$)[a]			
	Model	Mitochondria	GH	Mitochondria × GH
Body weight				
130 days	0.059	**0.003**	0.238	**0.034**
265 days	0.291	0.081	0.271	0.722
365 days	0.099	**0.023**	0.153	0.473
Specific gravity				
140 days	0.077	**0.007**	0.827	**0.064**
250 days	0.311	0.142	0.704	0.452
450 days	0.251	**0.018**	0.501	0.154

[a] Model: $y_{ijk} = mitgeno_i + ghgeno_j + mitgen_i \times ghgeno_j + e_{ijk}$.

Discussion

The mitochondrial genotype was associated with two traits, body weight and egg specific gravity, a trait which is highly associated with eggshell thickness. Association with body weight was significant at a relatively advanced age, despite an increase in variance with age. However, a similar trend was observed in younger chickens. The difference in weight gain between the two genotypic classes expressed as a percentage of the mean weight gain was 2% between day 0 and 130, 4% between day 130 and 265, and 50% between day 265 and 365. It indicates that the relative influence of the mitochondrial genotype increases with age, suggesting that the effect of mitochondrial genotype on growth may be an age-related phenomena as has been observed for many human mitochondrial disorders where the severity of symptoms increases with age (Wallace 1992). Age-related effects of mitochondrial variants had been attributed to the gradual loss of functional mitochondria throughout adult life. However, this may not be the case in chickens where traits were measured relatively early with respect to the natural life cycle. Rather the mechanisms by which mitochondrial functions interdigit with other metabolic enzymes is subject to changes throughout development. This is exemplified by our analysis of the interaction between the mitochondrial genotype and the growth hormone genotype, where interaction between the two genotypes was only significant at an early age.

In contrast to body weight, the average difference of egg specific gravity between the two mitochondrial classes remained similar throughout the three periods of measurements (1.1, 0.8, and 1.2, respectively). The coefficient of variance of this trait also increased with age and only the measurements taken at day 240 reached significance. As with body weight, significant interaction with the GH genotype was only observed at an early age.

The major determinant of specific gravity is eggshell thickness and hence the amount of calcium deposited in the eggshell. The turnover of calcium in laying hens is high, amounting to about 10% of the total body calcium for each egg laid (Soares 1983). Homeostasis of calcium is governed by at least four endocrine systems: calcitonin, parathyroid hormone, 1,25-dihydroxyvitamin D_3, and estrogen. Two key enzymes in the synthetic pathway of 1,25 dihydroxyvitamin D_3, the main hormone for calcium mobilization, are enzymes located in renal mitochondria, namely 25-OHD_3-1-α-hydroxylase (Gray et al. 1972) and 25-OHD_3-24 hydroxylase (Knutson and DeLuca 1974). Divergent selection lines of chickens for "thick" and "thin" eggshells has been shown to significantly lower the 25-$(OH)_2D_3$ levels in the strain selected for thin shells, raising the possibility of an involvement of mitochondrial variation (Soares et al. 1980). High plasma estradiol levels, presumably resulting in higher 25-$(OH)_2D_3$ levels, have also been associated with better eggshell quality (Grunder et al. 1983). Estradiol level may also be subject to modulation by mitochondrial activity, due to its involvement in steroid biosynthesis.

Although there is ample evidence to implicate mitochondrial genes as candidate genes responsible for genetic variations in eggshell quality as well as body growth, final evidence has to come from measuring mitochondrial functions. The particular marker mutation in the NADH dehydrogenase subunit IV gene occurs in a triplet coding for threonine, with *Msp*I$^+$ allele containing the triplet ACC and the *Msp*I$^-$ allele the triplet ACT. Both triplets code for threonine. The codon usage of ACC is three times higher than ACT in both humans and chickens, but the evolutionary significance of this difference is unknown (Anderson et al. 1981; Desjardin and Morais 1990). Although different codons may

Table 4. Least square means for body weight and egg specific gravity in dependence of GH and mitochondrial genotypes in strain 7

	GH and mitochondrial genotype[a]					
	$A1^+/A1^+$		$A1^+/A1^-$		$A1^-/A1^-$	
Trait	*Msp*I$^+$ (N = 58)	*Msp*I$^-$ (N = 134)	*Msp*I$^+$ (N = 41)	*Msp*I$^-$ (N = 82)	*Msp*I$^+$ (N = 5)	*Msp*I$^-$ (N = 21)
Body weight (g)						
130 days	1,270	1,304	1,272	1,287	1,148***	1,341***
265 days	1,736	1,777	1,705	1,743	1,626	1,752
365 days	1,759*	1,820*	1,733	1,777	1,584*	1,779*
Egg specific gravity						
240 days	85.2	85.3	84.4**	86.2**	82.0**	87.1**
350 days	81.7	81.8	80.9	82.1	81.2	84.0
450 days	79.0	79.3	77.7*	79.6*	74.5*	80.4*

[a] The observed distribution of genotypes did not significantly differ from the distribution expected from Hardy-Weinberg equilibrium for the GH genotypes and random association between GH and mitochondrial genotypes (χ^2 test, df = 4, $P < .5$). The significance between the trait means of the two mitocondrial genotypes within each GH genotypic class are indicated by $*P < .1$; $**P < .05$; and $***P < .01$.

affect transcription and/or translation, it is more likely that the particular *Msp*I RFLP is not directly responsible for the trait association, but rather that the association is due to linkage with an unknown mutation in another part of the mitochondrial genome.

One reason for identifying quantitative trait loci (QTL) is their potential usage for selecting at the DNA level. In the case of the mitochondrial marker selection for the *Msp*I$^-$ allele would be expected to result in an increased eggshell thickness, increased body weight and increased MD susceptibility (possibly MD-induced tumor growth). However, the present analysis indicates that the magnitude of the expected selection response may depend on the GH genotype and presumably also on variations in other genes present in the particular strain.

Conventional QTL mapping is conducted by crossing individuals or inbred lines and analyzing the cosegregation of phenotypes with marker genotypes in the F_2 generation (Lander and Botstein 1989). Since each offspring inherits a different admixture of alleles at different loci, such analyses only reveal QTL which have an effect regardless of the particular genetic background. The same is true for an association study of traits and markers in candidate genes within a strain by using analysis of variance. Such QTL may be economically most important, since they can be used for selection at the DNA level in most strains. However, the genetic architecture of quantitative traits is likely to be more complex and the effects of alleles in a gene have to be analyzed in the context of genetic variations in other genes. It is to be expected that most of the genetic variations for traits under intensive selection are of this type.

References

Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, and Young IG, 1981. Sequence and organization of the human mitochondrial genome. Nature 290:457–465.

Cole RK and Hutt FB, 1973. Selection and heterosis in Cornell White Leghorns: a review with special consideration of inter-strain hybrids. Anim Breed Abstr 41: 103–108.

Desjardins P and Morais R, 1990. Sequence and gene organization of the chicken mitochondrial genome: a novel gene order in higher vertebrates. J Mol Biol 212: 599–634.

Gavora JS, Kuhnlein U, and Spencer JL, 1989. Absence of endogenous viral genes in an inbred line of Leghorn chickens selected for high egg production and Marek's disease resistance. J Anim Breed Genet 106:217–224.

Gowe RS, Fairfull RW, McMillan I, and Schmidt GS, 1993. A strategy for maintaining high fertility and hatchability in a multiple-trait egg stock selection program. Poult Sci 72:1433–1448.

Gray RM, Omdahl JL, Ghazarin JG, and DeLuca HF, 1972. 25-hydroxycholecalciferol-1α-hydroxylase. Subcellular location and properties. J Biol Chem 247:7528–7532.

Grunder AA, Hollands KG, and Tsang CPW, 1983. Plasma estrogen, calcium and shell quality in two strains of White Leghorns. Poult Sci 62:1294–1296.

Gyllensten U, Wharton D, Josefsson A, and Wilson AC, 1991. Parental inheritance of mitochondrial DNA in mice. Nature 352:255–257.

Hartmann W, 1991. Genetic aspects of resistance to avian leucosis: an overview. Inform Tecnic Econ Agra 87A(2–3):171–189.

Hartmann W, Hagen VD, and Heil G, 1984. Genetic resistance to leucosis virus infection: effects on mortality and egg production in Leghorn hens. Br Poult Sci 106: 379–384.

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

Kimura M, 1955. Solution of a process of random genetic drift with a continuous model. Proc Natl Acad Sci USA 41:144–150.

Knutson JC and DeLuca HF, 1974. 25-hydroxyvitaminD_3-24 hydroxylase. Subcellular location and properties. Biochem 13:1543–1548.

Kuhnlein U, Gavora JS, Spencer JL, Bernon DE, and 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 77:26–32.

Kuhnlein U, Ni L, Weigend S, Gavora JS, Fairfull W, Zadworny D, 1997. DNA polymorphisms in the chicken growth hormone gene: response to selection for disease resistance and association with egg production. Anim Genet 28:116–123.

Kuhnlein U and Zadworny D, 1994. Disease resistance genetics: selection at the DNA level. In Proceedings of the 5th World Congress of Genetics Applied to Livestock Production, Guelph, Ontario, Canada. 21:249–256.

Lander ES and Botstein D, 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199.

Merriwether DA, Clark AG, Ballinger SW, Schurr TG, Soodyall H, Jenkins T, Sherry ST, and Wallace DC, 1991. The structure of human mitochondrial DNA variation. J Mol Evol 33:543–555.

Reed KC and DA Mann, 1985. Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acids Res 13: 7207–7221.

Soares JH Jr, 1984. Calcium metabolism and its control—a review. Poult Sci 63:2075–2083.

Soares JH Jr, Ottinger MA, Buss EG, and Kaetzel DM Jr, 1980. Plasma concentration of $1,25(OH)_2D_3$, estradiol and calcium in laying hens selected for differential egg shell quality [abstr]. Poult Sci 58:1662 (Abstract)

Wallace DC, 1992. Diseases of the mitochondrial DNA. Annu Rev Biochem 61:1175–1212.

Whittaker PA and Danks SM, 1978. Mitochondria: structure, function and assembly. New York: Longman.

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