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The role of growth hormone in lines of mice divergently selected on body weight

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Summary

An understanding of the physiological and genetic changes which determine the response to selection is critical for both evolutionary theory and to assess the application of new molecular techniques to commercial animal breeding. We investigated an aspect of physiology, growth hormone (GH) metabolism, which might *a priori* have been expected to play a large part in the response of mouse lines selected for high or low body weight. Disruption of endogenous GH or addition of exogenous GH had similar proportionate effects on body weight in both lines of mice (although differences in body composition arose) suggesting that neither the production of GH nor receptor sensitivity to GH had been altered as a result of selection. This supports a 'pleiotropic model' of the response to selection: that many genes with diverse metabolic roles all contribute to the divergent phenotype. This result has significant commercial implications as it suggests that artificial selection, transgenic technology and environmental manipulation may be synergistic rather than antagonistic strategies.

1. Introduction

The improvement of commercial livestock production has traditionally proceeded by breeding from those individuals with the most desirable phenotype. The responses to such selection are generally assumed to be polygenic, i.e. to involve changes of gene frequencies at a large number of loci. There is increasing interest in accelerating this process by directly manipulating individual genes with large effects on the desired trait. The technology now exists either to increase the activity of such genes (by inserting additional copies, e.g. Palmiter *et al.* 1982; Polge *et al.* 1989; Pursel *et al.* 1989) or to eliminate activity (by homologous recombination, e.g. Joyner 1991; Bradley *et al.* 1992). Another means of improving livestock production is by direct environmental physiological manipulation, for example by administration of exogenous growth factors such as growth hormone. This has become possible with the advent of technology capable of producing large amounts of recombinant proteins. The application of these technologies depends on their efficacy in combination: for example if selection for body weight has increased growth hormone (GH) production to a high level the insertion of additional GH genes may be futile; alternatively it may be pointless embarking on an (expensive) selection scheme if the response occurs predominantly by increasing GH production which may be artificially increased by recombinant technology.

This paper describes work investigating the role of GH in the response to selection by combining the strategies of selection, genetic manipulation, and environmental manipulation in order to examine their effects and interactions. This was accomplished by backcrossing the *little* (*lit*) gene into lines of mice divergently selected for body weight and injecting both parental and backcrossed lines with exogenous growth hormone. The *lit* gene is a recessive mutation which encodes a defective receptor of GH releasing factor; homozygotes fail to release significant levels of GH and are consequently dwarves (Eicher & Beamer, 1976; Phillips *et al.* 1982; Jansson *et al.* 1986). The lack of GH also results in reduced levels of its intermediate effector, insulin-like growth factor I (IGF-I). IGF-I has been associated with increased weight gain in the lines used here (McKnight & Goddard, 1989), in other mouse lines (Blair *et al.* 1989, 1990) and in other species such as sheep (Blair *et al.* 1990).

The experiment therefore addresses the following questions: (i) Is the difference between the High and

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Table 1. *Means (±standard deviations) of body weight at 4 and 7 weeks of age, weight gain from 4 to 7 weeks expressed in grams (g) or as a percentage of weight at 4 weeks (%). Means of carcass traits at 7 weeks of age expressed in grams (g) or percentage of dry weight (%). Standard errors are averaged standard errors of group means*

	High				Low				
	+/+		*lit/lit*		+/+		*lit/lit*		
	GH	Saline	GH	Saline	GH	Saline	GH	Saline	
*n**	14	15	14	15	14	14	15	15	S.E.
4 week wt (g)	21·6±5·6	21·0±4·9	10·6±1·4	10·6±1·0	11·6±1·8	11·6±1·5	5·7±0·8	5·9±0·9	0·58
7 week wt (g)	44·2±3·3	39·5±3·4	24·1±2·7	17·6±1·7	18·3±1·5	17·2±1·0	11·0±1·1	8·4±1·0	0·51
Gain (g)	22·5±2·8	18·6±3·5	13·5±1·9	7·0±1·1	6·7±1·0	5·5±0·8	5·3±0·8	2·5±0·5	0·41
Gain (%)	114±43	95±34	129±20	66±12	60±16	49±13	95±21	45±18	5·8
Water (g)	29·1	26·2	14·7	9·7	11·9	11·2	7·0	5·1	0·32
Fat (g)	3·6	3·1	3·3	3·7	1·4	1·5	1·0	1·2	0·17
Protein (g)	8·5	7·8	4·2	2·9	3·6	3·4	2·1	1·5	0·07
Ash (g)	1·4	1·3	0·8	0·6	0·6	0·6	0·4	0·3	0·16
Fat (%)	26	24	39	50	24	26	28	38	1·3
Protein (%)	60	61	49	39	62	62	57	49	1·0
Ash (%)	10	11	9	8	11	11	11	10	0·2

* Number of animals, and records, of body weights, water weights and gains. For other traits there were four samples of pooled mice within each group.

Low selected lines solely due to differences in the production of, or sensitivity to, GH and/or its mediators such as IGF-I?; if so both lines would grow at the same rate when homozygous *lit/lit*. (ii) If the differences cannot be attributed solely to GH, does the *lit* gene have the same effect in both genetic backgrounds; in genetic terms, is its action purely additive or do significant epistatic effects arise? (iii) Has the sensitivity to GH been altered by selection as revealed by a differential response to exogenous GH? This also investigates the influence of the correct expression and reception of GH which is of considerable practical importance; for example in many experiments additional copies of GH genes have been inserted into animals resulting in the continuous release of GH in contrast to the usual situation *in vivo* where GH production may be pulsatile and vary between sexes. GH receptor levels, at least in rats, may be partly regulated by circulating GH (Kelly *et al.* 1991), constituting another physiological variable which may have been altered by selection.

2. Materials and Methods

The origin and selection of the mouse lines used in this experiment (the 'P' lines) have been described by Sharp *et al.* (1984) and Beniwal *et al.* (1992*a*) and the introduction of the *lit* gene by Bootland *et al.* (1991*a*). Briefly, the lines were derived from a crossbred base and selected for 20 generations on estimated lean mass at 10 weeks of age (using an index of body weight and gonadal fatpad weight), and for a further 23 generations on body weight at 10 weeks of age. At the time of this experiment they differed 2·5- to 3-fold in weight at 6 and 10 weeks of age. The *lit* gene (on a C57BL/J background) was backcrossed into these lines for three generations, giving an average of 93% parental line alleles at loci unlinked to the *lit* gene.

Recombinant bovine GH, a gift from American Cyanamid Co., had previously been shown to be biologically active in mice (Bootland *et al.* 1991*b*). It was administered as a 1·8 mg ml^{-1} solution in isotonic bicarbonate buffer saline pH 9·4 in a single daily dose of 9 $\mu g\ g^{-1}$ body weight; controls received the appropriate volume of the same bicarbonate saline. The GH solution was made up directly from the lyophilized form when required. It was never stored in this form for more than 26 h (at 4 °C) and during the third week of the experiment it was made up fresh each day. Injections were subcutaneous and were performed in the period 130 to 45 min prior to the start of the dark period.

Growth between 4 and 7 weeks of age was recorded in males. Four lines were investigated: the High and Low selected lines, and the same lines made homozygous for the *lit* gene as described above. Matings were made within lines so the *lit* gene was not segregating within families. Contemporaneous matings were set up in a 14 h light, 10 h dark cycle. Births occurred over a 5-day period and all litters were weaned on the same day (at age 26±2 days). Mice were provided with BP expanded diet no. 1 (Special Diets Services, Witham, Essex, UK) *ad libitum* in the standard cubed form in the cage food hoppers. It was also provided as a powder mixed with water and placed in a Petri dish on the cage floor. The latter food source was necessary as some *lit/lit* mice were very small at

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Table 2. *Main effects and interactions (±standard errors) for body weight (BW), gain, lean mass and % fat at 7 weeks of age, from REML analysis*

	BW (g)	Gain (g)	Lean mass (g)	% Fat
			Untransformed	
Main effects				
Background (High-Low)	17·9±0·6***	10·2±0·5***	3·20±0·06***	5·5±1·1***
lit gene (*lit/lit* − +/+)	−14·7±0·6***	−5·9±0·5***	−3·15±0·06***	13·8±1·1***
Treatment (GH-saline)	3·7±0·3***	3·6±0·2***	0·68±0·06***	−5·5±1·1***
Interactions				
lit × background	−6·7±0·9***	−3·8±0·7***	−1·42±0·08***	5·9±1·5***
lit × treatment	1·0±0·6	1·1±0·5*	0·23±0·08**	−5·0±1·5**
Background × treatment	1·8±0·6**	1·6±0·5***	0·32±0·08***	0·4±1·5
lit × background × treatment	0·3±0·9	0·3±0·8	0·04±0·12	−1·3±2·1
			Natural log. transformed	
Main effects				
Background (High-Low)	0·82±0·03***	1·08±0·05***	0·76±0·02***	0·14±0·04***
lit gene (*lit/lit* − +/+)	−0·67±0·03***	−0·60±0·05***	−0·77±0·02***	0·42±0·04***
Treatment (GH-saline)	0·19±0·01***	0·51±0·07***	0·20±0·02***	−0·14±0·04***
Interactions				
lit × background	−0·05±0·04	−0·11±0·07	−0·07±0·03**	0·15±0·06**
lit × treatment	0·10±0·03***	0·26±0·07***	0·13±0·03***	−0·13±0·06*
Background × treatment	0·02±0·03	−0·02±0·07	0·02±0·03	0·04±0·06
lit × background × treatment	0·00±0·04	−0·02±0·08	0·00±0·04	−0·05±0·08

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

weaning and might have been unable to reach food in the standard hoppers.

Families were split across treatments (GH or saline) and 15 mice assigned to each of the 8 groups represented in Table 1. All were weighed and received the first injection on the same day (at age 28±2 days). Each cage contained six mice with at least one from each line, and was assigned a single treatment (GH or saline) to reduce the risk of errors. Ten cages were necessary for each treatment; these were numbered 1–10 and injected in numerical sequence with the sequence rotated daily to minimize circadian differences in murine sensitivity to exogenous GH (i.e. cage 1 was the first cage injected on day 1, cage 2 on day 2, etc, reverting to cage 1 on day 11 and so on). Saline and GH cages were injected alternately. Mice were weighed three times each week (Mon., Wed., Fri.) and injected with a daily dose calculated from the last available weight. After the experiment the mice were weighed, killed and freeze dried.

Mice within each of the eight groups (Table 1) were sorted into four samples of three individuals with cages and families split across samples. The composition of each sample was determined chemically for fat, nitrogen (N) and ash content. Protein content was estimated in the standard manner as 6·25 N.

Body weight, gain, lean mass and fat content at 7 weeks of age were analysed by restricted maximum likelihood (REML) using the Genstat statistics package (Genstat 1988). The model fitted the components shown in Table 2 and, where appropriate, the (random) effects of family, cage, sequence of injection and batch of freeze drying. Significance of effects was determined using a *t* test with D.F. = 24 (lean mass, % fat) or D.F. > 70 (body weight and gain).

3. Results

There were no obvious deleterious effects of the injections. Four mice were removed from the experiment (and from subsequent analysis) for the following reasons: two were misclassified females, one was bitten by its cage mates, and one was found dead on day 4.

Growth over the period is shown on Fig. 1. A summary of body weights, weight gains and carcass composition at 7 weeks of age is given in Table 1. As is typical in such analyses, the percentage body

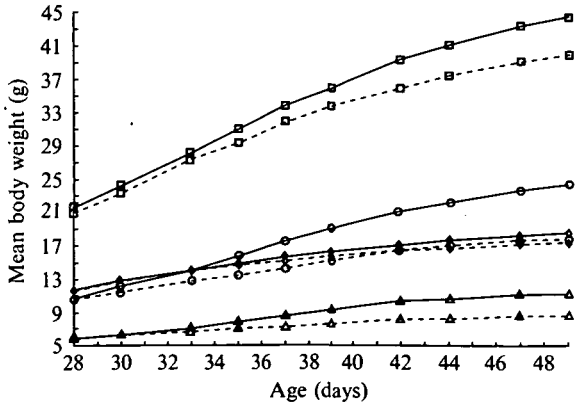

Fig. 1. Growth of mouse lines injected with exogenous growth hormone (——) and the appropriate controls (– – –) injected with saline. □, High +/+; ◇, Low +/+; ○, High *lit/lit*; △, Low *lit/lit*.

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composition does not sum to exactly 100% since the components are estimated independently. As expected, genetic background, GH injection and the *lit/lit* genotype all affected body weights and gains. In addition the *lit/lit* genotype had large effects on body composition, notably in the High genetic background where the *lit/lit* genotype elevated fat from 24 to 50%.

Estimates of these effects from the REML analysis are given in Table 2. In REML analysis the comparisons between treatments both between and within litters are weighted appropriately (Patterson & Thompson, 1971). Comparisons between genetic backgrounds and the *lit/lit* genotype were between litters so the associated standard errors are larger than those between treatment which was within litters. Analyses are given for weight at 7 weeks, weight gain, protein weight ('lean mass') and % fat. These key parameters describe growth and other traits are related to them (for example % protein is almost complementary to % fat).

4. Discussion

As expected, genetic background, GH injection and the *lit/lit* genotype all affected body weights and gains (Fig. 1, Table 1). The High background and GH injection increased 7-week body weight by an average of 17·9 and 3·7 g respectively, while the *lit/lit* genotype decreased it by an average of 14·7 g (Table 2). REML analysis of untransformed (linear scale; Table 2) body weights revealed a significant interaction between the *lit/lit* genotype and genetic background, the *lit* gene reducing body weight more in the High line; however this interaction was small and non-significant on log-transformed data, showing that the *lit* gene had a similar *proportionate* effect in both genetic backgrounds. Thus there is an approximate 2-fold difference ($e^{0.82}$) between the High and Low lines in body weight and a reduction of approximately 50% ($e^{-0.67}$) due to the *lit/lit* genotype. Since these effects are additive there is a 4-fold difference in body weight between the High/+ and Low/*lit* lines. The 50% reduction in body weight attributable to the *lit/lit* genotype is similar to that observed for the *lit/lit* genotype in unselected genetic backgrounds (Eicher & Beamer, 1976) and in unselected lines of mice where GH production has been eliminated by genetic ablation of GH secreting cells (Behringer *et al.* 1988).

There was no apparent interaction between genetic background and the *lit* gene which implies the effects of the GH axis had not been altered over the course of selection. These conclusions were reached from analysis of log-transformed data; this reduces scale effects and so is more appropriate than linear data for the analysis of body weights which may differ upto 5-fold between groups (Table 1).

Similar results to those obtained for body weight were obtained for lean mass at 7 weeks of age (Table 2). One exception is the presence of a significant interaction between the *lit/lit* genotype and the genetic background which remains even after transformation of the data. This may have arisen as a result of a repartition of growth away from lean and into fat. The similar magnitude of main effects and interactions on both body weight and lean mass are in agreement with previous analyses showing that the two traits had high phenotypic and genetic correlations (both over 0·9, Beniwal *et al.* 1992*b*) and that the amount of fat in the carcass could be genetically altered with little effect on the underlying lean mass (Hastings *et al.* 1991).

As expected, there was a significant interaction between the effects of GH and the presence or absence of the *lit* gene: exogenous GH increased weight by 35% in the *lit/lit* genotype (which lacks significant levels of endogenous GH) and by around 10% in the +/+ genotype. This latter result is in agreement with a previous study of these lines (Bootland *et al.*, 1991*b*) which reported increased body weights in High and Low +/+ genetic backgrounds of 6 or 15% depending on whether response was expressed as body weight or weight gain respectively. Similar results were obtained by Nagai *et al.* (1990) who crossed male mice, transgenic for a rat GH construct, with females from lines selected on body weight, nursing ability, or unselected controls; the additional GH produced by the transgenes increased body weight of their offspring by about 16% at 6 weeks of age and showed no interaction with maternal genetic background.

The genetic background had only a small effect on fat content, the High background increasing % fat by about a sixth. This confirms previous studies showing only a small correlated change in fat content associated with the 2·5-fold divergence in body weight (Hastings & Hill, 1989). However, the *lit* gene greatly increased the proportion of fat, doubling it in the High genetic background and increasing it by around a half in the Low genetic background in the absence of exogenous GH; in this trait there is a large interaction between the *lit* gene and the genetic background on both linear and logarithmic scales. Exogenous GH reduced this extra fat attributable to the *lit/lit* genotype by about one third in both backgrounds. The effects of the *lit/lit* genotype on fat content are probably explicable by the known lipolytic effects of GH (Fielder & Talamantes, 1992). However the reason for its interaction with genetic background remains unexplained.

There was no interaction between genetic background and the effects of exogenous GH in any trait (provided body weight and lean mass are analysed on transformed data). This implies that the sensitivity to GH had not been altered and, indirectly, that the sensitivity or numbers of hormone receptors had not changed over the course of selection.

Pidduck & Falconer (1978) performed a similar experiment by backcrossing the Snell dwarf (*dw*) gene

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into lines divergently selected on body weight. Their data were similar to ours in suggesting that differences in GH metabolism were not the sole cause of the observed divergence in body weight, but dissimilar in that a small but significant interaction occurred between the *dw/dw* genotype and genetic background. One complication of their methodology lay in their use of the *dw* gene (*lit* was not then available): the *dw/dw* genotype lacks not only GH but the anterior pituitary hormones PRL, TSH, FSH and LH (Cheng *et al.* 1983). In contrast, the effects of the *lit* gene appear restricted to the disruption of GH release (Clarke & Robinson, 1985; Jansson *et al.* 1986; Behringer *et al.* 1990). Pidduck & Falconer (1978) further suggest that sensitivity to GH may have decreased in the Low line but not in the High or Control lines. Sensitivity in the form of a dose/response study did not form part of our protocol as artificial elevation of GH is usually massive whether achieved by injection of GH or by endogenous transgenes (for example transgenes may increase GH levels several hundred-fold; Palmiter *et al.* 1983). Also, it is not clear how changes to a single daily dose of *bovine* GH should be interpreted biologically. Our results and those of Pidduck & Falconer are similar in two important respects: that GH is not the only factor causing differences in growth rate in response to artificial selection, and that little or no disproportionate response had occurred in this area of metabolism. Selection may still act on individual components of GH metabolism, and Salmon *et al.* (1988) and Winkelman & Hodgets (1992) found evidence of selection at the GH coding locus in several lines of mice selected for increased body weight, including one derived from that studied by Pidduck & Falconer (1978).

Many studies have identified physiological traits which may have been altered as a result of divergent selection (e.g. O'Sulliven *et al.* 1986; McKnight & Goddard, 1989; Blair *et al.* 1990) although such studies are frequently unreplicated so the effects of selection cannot formally be distinguished from the spurious effects of genetic drift. In addition, such studies cannot separate cause from effect; for example, the changes in enzyme activity associated with obesity caused by divergent selection (Asante *et al.* 1989, Hastings & Hill, 1990) are similar to those observed when obesity is due to a single gene (Bulfield, 1972). Such studies identify the *mechanism* by which the phenotype is altered (and thus may identify suitable genes for disruption by homologous recombination) but give few clues as to the actual genotypic changes which have occurred. The approach described here differs in using direct genetic manipulation so is experimental rather than purely observational. Such studies may, by eliminating specific portions of metabolism, provide insights into their relative importance in response and to the epistatic, pleiotropic and dominance effects of the underlying genes.

In this particular case of weight in mice, it suggests that a part of metabolism which might *a priori* be expected to have been subjected to a disproportionate selection pressure, appears not to have responded. This may be due to a lack of additive genetic variance but is unlikely, because such variance has been demonstrated previously for its effector IGF-I (Blair *et al.* 1990), and because genetic variance in GH appears not to have been utilized in response to selection on body weight when such variance is present as a result of transgenes (Sabour *et al.* 1991). Furthermore, a gene causing increased growth in mice is associated with a *decreased* level of circulating GH (Medrano *et al.* 1991), but with an increased level of IGF-I. This latter result illustrates a point of general biological relevance to the response to selection: that critical areas of metabolism are typically tightly regulated by feedback mechanisms and response may therefore be more likely in the mediators and effectors of such systems. If we conclude that disproportionate pressures did not act on GH metabolism, this strongly supports the pleiotropic model of genetic divergence: that many genes with diverse metabolic roles all contribute to the divergent phenotype.

The use of new technologies to manipulate growth in commercial species depends on their efficacy in combination. The results described here demonstrate two principles relevant to their application. Firstly the lack of interaction between genetic background, the *lit* gene and GH injection (with the exception of a significant but relatively small *lit* by GH interaction) on the trait under selection (log-transformed BW, Table 2) suggests that animal breeding, transgenic technology and environmental manipulation may be synergistic approaches rather than antagonistic. Secondly, investigations using model species like the mouse may identify unpredicted consequences of genetic manipulation, in this case the large interaction between the *lit* gene and genetic background on carcass fat content.

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References

Asante, E. A., Hill, W. G. & Bulfield, G. (1989). Analysis of lines of mice selected for fat content. 1. Correlated responses in the activities of NADPH-generating enzymes. *Genetical Research* **54**, 155–160.

Behringer, R. R., Lewin, T. M., Quaife, C. J., Palmiter, R. D., Brinster, R. L. & Dercole, A. J. (1990). Expression of insulin-like growth factor-I stimulates normal somatic growth in growth hormone-deficient transgenic mice. *Endocrinology* **127**, 1033–1040.

Behringer, R. R., Mathews, L. S., Palmiter, R. D. & Brinster, R. L. (1988). Dwarf mice produced by genetic ablation of growth hormone-expressing cells. *Genes & Development* **2**, 453–461.

Beniwal, B. K., Hastings, I. M., Thompson, R. & Hill, W. G. (1992*a*). Estimation of changes in genetic parameters in selected lines of mice using REML with an animal model. I. Lean mass. *Heredity* **69**, 352–360.

Beniwal, B. K., Hastings, I. M., Thompson, R. & Hill, W. G. (1992*b*). Estimation of changes in genetic parameters in selected lines of mice using REML with an animal model. II. Body weight, body composition and litter size. *Heredity* **69**, 361–371.

Blair, H. T., McCutcheon, S. N., Mackenzie, D. D. S., Gluckman, P. D., Ormsby, J. E. & Brier, B. H. (1989). Responses to divergent selection for plasma concentrations of insulin-like growth factor-1 in mice. *Genetical Research* **53**, 187–191.

Blair, H. T., McCutcheon, S. N. & Mackenzie, D. D. S. (1990). Components of the somatotropic axis as predictors of genetic merit for growth. *Proceedings of the 4th World Congress Applied to Livestock Production* **XVI**, 246–255.

Bootland, L. H., Hill, W. G. & Hastings, I. M. (1991*a*). Production of high and low body weight *lit/lit* dwarves. *Mouse Genome* **89**, 564.

Bootland, L. H., Hill, W. G. & Sinnett-Smith, P. A. (1991*b*). Effects of exogenous growth hormone on growth and composition in genetically selected mice. *Journal of Endocrinology* **131**, 19–24.

Bradley, A., Hasty, P., Davis, A. & Ramirez-Solis, R. (1992). Modifying the mouse: design and desire. *Biotechnology* **10**, 534–538.

Bulfield, G. (1972). Genetic control of metabolism: enzyme studies of the *obese* and *adipose* mutants in the mouse. *Genetical Research* **20**, 51–64.

Cheng, T. C., Beamer, W. G., Phillips, J. A., Bartke, A., Mallonee, R. L. & Dowling, C. (1983). Etiology of growth-hormone deficiency in little, ames, and snell dwarf mice. *Endocrinology* **113**, 1669–1678.

Clark, R. G. & Robinson, I. C. (1985). Effects of a fragment of human growth hormone-releasing factor in normal and little mice. *Journal of Endocrinology* **106**, 1–5.

Eicher, E. M. & Beamer, W. G. (1976). Inherited ateliotic dwarfism in mice. *Journal of Heredity* **67**, 87–91.

Fielder, P. J. & Talamantes, F. (1992). The insulin-like effects of mouse growth-hormone on adipose-tissue from virgin and pregnant mice. *Metabolism – Clinical and Experimental* **41**, 415–419.

Genstat 5 Committee (1988). *Genstat 5 Reference Manual.* Oxford, UK: Oxford University Press.

Hastings, I. M. & Hill, W. G. (1989). A note on the effects of different selection criteria on carcass composition in mice. *Animal Production* **48**, 229–233.

Hastings, I. M. & Hill, W. G. (1990). Analysis of lines of mice selected for fat content. 2. Correlated responses in the activities of enzymes involved in lipogenesis. *Genetical Research* **55**, 55–61.

Hastings, I. M., Yang, J. & Hill, W. G. (1991). Analysis of lines of mice selected on fat content. 4. Correlated responses in growth and reproduction. *Genetical Research* **58**, 235–259.

Jansson, J. O., Downs, T. R., Beamer, W. G.& Frohman, L. A. (1986). Receptor-associated resistance to growth hormone-releasing factor in dwarf little mice. *Science* **232**, 511–512.

Joyner, A. L. (1991). Gene targetting and gene trap screens using embryonic stem cells – new approaches to mammalian development. *BioEssays* **13**, 649–656.

Kelly, P. A., Djiane, J., Postel-Vinay, M. C. & Edery, M. (1991). The prolactin/growth hormone receptor family. *Endocrine Reviews* **12**, 235–251.

McKnight, B. J. & Goddard, C. (1989). The effect of food restriction on circulating insulin-like growth factor-I in mice divergently selected for high or low protein of fat to body mass ratios. *Comparative Biochemistry and Physiology* **92**, 656–569.

Medrano, J. F., Pomp, D., Sharrow, L., Bradford, G. E., Downs, T. R. & Frohman, L. A. (1991). Growth hormone and insulin-like growth factor-1 measurement in high growth (hg) mice. *Genetical Research* **58**, 67–74.

Nagai, J., Davis, G. & Lin, C. Y. (1990). Growth of mice produced by males with or without the rat growth-hormone transgene. *Canadian Journal of Animal Science* **70**, 979–982.

O'Sulliven, D., Millard, W. J., Badger, T. M., Martin, J. B. & Martin, R. J. (1986). Growth hormone secretion in genetic large (LL) and small (SS) rats. *Endocrinology* **119**, 1948–1953.

Palmiter, R. D., Brinster, R. L., Hammer, R. E., Trumbauer, M. E., Rosenfeld, M. G., Birnberg, N. C. & Evans, R. M. (1982). Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* **300**, 611–615.

Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E. & Brinster, R. L. (1983). Metallothionein-human GH fusion genes stimulate growth in mice. *Science* **222**, 809.

Patterson, H. D. & Thompson, R. (1971). Recovery of inter-block information when block sizes are unequal. *Biometrika* **58**, 545–554.

Phillips, J. A. I., Beamer, W. G. & Bartke, A. (1982). Analysis of growth hormone genes in mice with genetic defects of growth hormone expression. *Journal of Endocrinology* **92**, 405–407.

Pidduck, H. G. & Falconer, D. S. (1978). Growth hormone function in strains of mice selected for large and small size. *Genetical Research* **32**, 195–206.

Polge, E. J. C., Barton, S. C., Surani, M. A. H., Miller, J. R., Wagner, T., Rottman, F., Camper, S. A., Elsome, K., Davis, A. J., Goode, J. A., Foxcroft, G. R. & Heap, R. B. (1989). Induced expression of a bovine growth hormone construct in transgenic pigs. In *Biotechnology in Growth Regulation* (ed. R. B. Heap, C. G. Prosser and G. E. Lamming). London: Butterworths.

Pursel, V. G., Miller, K. F., Bolt, D. J., Pinkert, C. A., Hammer, R. E., Palmiter, R. D. & Brinster, R. L. (1989). Insertion of growth hormone genes into pig embryos. In *Biotechnology in Growth Regulation* (ed. R. B. Heap, C. G. Prosser and G. E. Lamming). London: Butterworths.

Sabour, M. P., Ramsey, U. & Nagai, J. (1991). Decreased frequency of the rat growth-hormone transgene in mouse-populations with or without selection for increased adult body-weight. *Theoretical and Applied Genetics* **81**, 327–332.

Salmon, R. K., Berg, R. T., Yeh, F. C. & Hodgetts, R. B. (1988). Identification of a variant growth hormone haplotype in mice selected for high body weight. *Genetical Research* **52**, 7–15.

Sharp, G. L., Hill, W. G. & Robertson, A. (1984). Effects of selection on growth, body composition and food intake in mice. I. Responses in selected traits. *Genetical Research* **43**, 75–92.

Winkelmann, D. C., & Hodgetts, R. B. (1992). RFLPs for somatotropic genes identify quantitative trait loci for growth in mice. *Genetics* **131**, 929–937.