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Direct and Correlated Responses to Short-term Selection for 8-week Body Weight in Lines of Transgenic (oMt1a-oGH) Mice

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The objective of this experiment was to evaluate the results of selection for increased 8-week body weight in lines of mice with or without a sheep metallothionein 1a sheep growth hormone (oMt1a-oGH) transgene in two genetic backgrounds. The transgene was introgressed into two lines of mice which had previously either been selected for rapid growth or randomly selected. Selection was practiced within families of full-sibs for seven generations. Selection was effective in increasing 8-week body weight in all non-transgenic lines and in some of the transgenic lines. The initial transgene frequency of 0.5 increased to about 0.6 in the lines with random selection background, but decreased to less than 0.2 in those lines from the selected background. Correlated responses in other growth and fitness traits were observed in some lines, and when present were chiefly in the desired direction. It was concluded that selection in the transgenic lines was successful, although the response was dependent on the genetic background. Realized response and realized heritability for 8-week body weight were lower in the transgenic than in the non-transgenic lines, but no significant differences were found between the selected and unselected background, nor was there any significant interaction. The lower response in the transgenic lines may have been due to reduced prenatal survival of transgenic embryos.

Introduction

Large-scale use of transgenics in animal breeding plans is not yet in effect, although incorporation of foreign DNA into commercial livestock species has already been achieved (Rexroad, 1992). One problem has been that

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most transgene constructs developed for livestock have been poorly regulated. Another major difficulty lies in the method of gene transfer most commonly used, namely, microinjection. The number of transgenic individuals produced by microinjection is limited, and the site of insertion of the transgene construct is usually distinct for each transgenic founder animal. In addition, each line formed is partially inbred because all individuals descend from the same founder animal. Aside from specific expected benefits to production traits or disease resistance, a transgene should not have undesirable effects on fitness and should be able to be regulated. The activity of a transgene is affected both by its insertion site (Al-Shawi *et al.*, 1990) and by the background of the line in which the transgene is to be inserted (Eisen *et al.*, 1995; Siewerdt *et al.*, 1998).

The introduction of the sheep metallothionein 1a sheep growth hormone transgene (oMt1a-oGH) into the murine genome and its regulation are well documented (Shanahan *et al.*, 1989; Oberbauer *et al.*, 1992). The oMt1a-oGH transgene can be activated by adding supplementary zinc to the drinking water. Levels of circulating growth hormone become highly elevated upon activation of the transgene, but return to basal levels within 24 h after withdrawal of the zinc sulphate (Shanahan *et al.*, 1989). The oMt1a-oGH transgene incorporated into mice has been shown to increase growth rate, reduce fat content, and apparently has few unfavourable fitness problems (Pomp *et al.*, 1992; Eisen *et al.*, 1995; Murray and Pomp, 1995; Clutter *et al.*, 1996; Siewerdt *et al.*, 1998). The type of gene action of the oMt1a-oGH transgene was determined by Siewerdt *et al.* (1998). Dominance was the predominant form of action of the transgene on body weights and organ weights, although some differences were found according to the selection background of the lines into which the transgene was introgressed.

Sabour *et al.* (1991) reported the sole selection experiment with transgenic lines found in the literature, with lines carrying the rat growth hormone transgene. To our knowledge the present report is the first selection experiment on lines of mice carrying the oMt1a-oGH transgene insert. This paper reports the results of seven generations of selection for increased 8-week body weight in transgenic and non-transgenic lines of mice with different selection backgrounds. Direct and correlated responses to selection and patterns of change in frequency of the transgene are described.

Materials and Methods

Ten male mice from the MG101 line (Shanahan *et al.*, 1989) were tested for homozygosity for the oMt1a-oGH transgene insert. The structure considered an 'allele' of the oMt1a-oGH in the MG101 line consists of five copies of the insert (Shanahan *et al.*, 1989). Line MG101 originated due to an unequal crossover event in the original line, which carried 43 copies of the insert.

The transgenic males were mated to virgin females of a high growth line (M16), which has a history of 27 generations of selection for increased postweaning weight gain from 3 to 6 weeks (Eisen, 1975), and were also mated to females of a randomly selected control line (ICR) from which M16 originated. Hemizygous mice in the F1 were assumed to be transgenic and were reciprocally backcrossed, respectively, to the M16 and ICR lines. Tail-clips were obtained from backcross mice at 6 weeks of age as a source of DNA for a PCR analysis (Pomp and Murray, 1991). Mice testing positive for the presence of the oMt1a-oGH transgene insert were designated as founders of the TM and TC lines, respectively, from the crosses with M16 and ICR lines. Non-transgenic mice formed generation 0 of the NM and NC lines, in that order. A control line (CC) was formed from the same pool that originated the NC line. One male and one female from each litter in the CC line were selected randomly as parents of the next generation. The entire procedure was repeated after 4 weeks to form a second replicate. Each replicated line of the selection treatments comprised approximately 16 pairs of sires and dams.

Selection was practiced within families for seven generations on lines TM, TC, NM and NC. The selection criterion was large 8-week body weight. The heaviest male and female from each full-sib family were selected as parents for the next generation. Mice were pair-mated randomly but sib matings were not allowed. Line names were labelled with their corresponding replication number (1 or 2). Mice were fed *ad libitum* Purina Mouse Chow 5015 (17.5% crude protein, 11.0% fat, 4.35 kcal g^{-1} gross energy, 102.2 ppm zinc) from PMI Feeds, Inc. (St Louis, Missouri), and received tap water from mating until weaning at 3 weeks of age. From 3 to 8 weeks of age mice received Purina Lab Chow 5001 (23.4% crude protein, 4.5% fat, 4.00 kcal g^{-1} gross energy, 70.0 ppm zinc) and 25 mM zinc sulphate in distilled drinking water. Temperature (22°C) and humidity (55%) were kept constant in the laboratory. A light regime consisting of 12 h of light and 12 h of darkness (0700–1900) was used.

Data on body weights were collected at 3, 6 and 8 weeks of age (BW3, BW6 and BW8, respectively). Body weight gains were calculated for the periods between 3 and 6 weeks (GAIN36) and 6 and 8 weeks (GAIN68). Matings were done at about 10 weeks of age and cohabitation lasted for 17 days. Litters were standardized to eight pups or less within 24 h of birth. If less than eight live pups were born in a litter, crossfostering was used, provided that pups from other litters of the same line, born on the same day, were available. Litter sizes and dead pup numbers were recorded.

Body weight and weight gain data were analysed using PROC MIXED of SAS (SAS Institute, 1992). A mixed model that included the fixed effects of line, generation and sex, their interactions and the random effects of litter, nested within interaction of line and generation was fitted to the data of each replication. Replications were assumed to be random. Least-squares line means for each trait were expressed as deviations from the CC line, and were

compared in the form of three orthogonal contrasts: transgenic versus non-transgenic lines ([TM+TC−NM−NC]/2), selected versus control background ([TM−TC+NM−NC]/2), and interaction between effects of the transgene and selection background ([TM−TC−NM+NC]/2). Realized heritabilities for BW8 were estimated by regressing the generation means of BW8 on the cumulative selection differentials (Hill, 1972). The selection differentials were weighted by the number of progeny with a record for BW8 produced by each individual. Realized heritabilities for individual selection were obtained by multiplying the within-family selection heritabilities by the factor $(1-t) \times (1-r)^{-1}$, where t is the full-sib intraclass correlation for the specific line and replication and $r = 0.5$ (Falconer and Mackay, 1996). The analysis assumes that there is no line–environment interaction. In this situation the randomly selected control populations account accurately for any environmental trends present (Muir, 1986). Correlated responses were obtained on BW3, BW6, GAIN36 and GAIN68 by regressing their corresponding deviations from CC line means on generation number.

Fitness traits measured on dams were: cohabitation to littering interval (CLI), litter size (LS), and preweaning mortality (MORT), defined as 100 × (number of live pups at weaning) × (number of pups after standardization at day 1)$^{-1}$. The proportion of infertile matings was also obtained. Two fitness indexes were defined as FI_1 = (litter size) × (proportion of fertile matings) × (proportion of preweaning pup survival) and $FI_2 = 0.8 \times FI_1 - 0.2 \times CLI$. The inclusion of CLI in the second fitness index favours the females that successfully mated and produced a litter in a shorter period of time. Data on fitness traits were analysed with a linear model including the effects of generation and replication. Each trait had its generation means for lines TM, TC, NM and NC expressed as deviations from corresponding mean for the line CC. Regression of these deviations over generation number provided estimates of correlated responses in fitness traits.

The regression coefficients for direct and correlated responses to selection in growth and fitness data were compared with the same three orthogonal contrasts used for mean body weights: transgenic versus non-transgenic lines, selected versus control background, and their interaction. When the interaction was significant, a further decomposition compared line TM with NM (effect of the transgene in the selected background) and line TC with NC (effect of the transgene in the control background).

In generations one through seven, tail-clips were collected on mice of lines TM and TC at 6 weeks of age as a source of DNA for PCR analyses. DNA samples from generations 2–6 were probed with a semi-quantitative PCR analysis (Schrenzel and Ferrick, 1995), which allows distinction between hemizygous (T/−) and homozygous (T/T) transgenics. The frequency of the transgene insert was calculated by allele counting. A qualitative PCR was run on DNA samples from generations 1 and 7. This analysis only makes distinction between non-transgenics (−/−) and transgenic mice. Since homozygous and hemizygous transgenics could not

be distinguished, the frequencies of the transgene were calculated assuming the empirical genotypic ratios found by Siewerdt *et al.* (1998), which differed significantly from the genotypic proportions assumed when Hardy–Weinberg equilibrium holds. No PCR results were available for mice from replication 1 in generation 2, because the DNA samples were degraded.

Results

In the backcross generation, an overall percentage of 45.4% of transgenics was obtained. This percentage differs from 50% ($P<0.01$). In replications 1 and 2 percentages of 48.3 and 40.4% ($P<0.01$) of transgenics were observed, respectively. There was an under-representation of transgenic males (40.7%, $P<0.01$), but not of transgenic females (48.0%). With both selection backgrounds the percentage of transgenics was different from the 50% expected from the theoretical 1:1 ratio, 45.4% ($P<0.01$) in line ICR, and 44.1% ($P<0.01$) in line M16.

In generation 0, no significant line differences were found for percentage of infertile matings and percentage preweaning pup mortality, the overall means being 9.0% and 2.9%, respectively. However, the analysis of variance showed significant line differences for CLI ($P<0.05$) and LS ($P<0.01$). Mating between hemizygous transgenic mice (TC, TM) had a longer CLI ($P<0.01$) and a smaller LS ($P<0.01$) than non-transgenic mice (Table 16.1). The history of selection for high postweaning gain in the NM and TM lines explains the larger litter sizes in these lines compared with the control background lines (NC and TC), because selection for high postweaning gain led to a positive correlated response in litter size (Eisen *et al.*, 1973). Selection background had no significant effect on CLI. No significant interaction between the effects of selection background and transgene was detected for CLI and for LS. The CC and NC lines had similar means for these traits, as expected since no selection had yet occurred in NC.

The estimated frequencies of the transgene insert are presented in Fig. 16.1. In both replications of line TM the frequency declined from the initial value of 0.5. In line TM2 the frequency was below 0.05 as of generation 7, and there were no homozygous transgenic individuals in generations 6 and 7. In the TC line, the frequency of the transgene rose to values around 0.6 beginning in generation 3, except for small fluctuations observed on TC1 in generation 7 and on TC2 in generation 5.

All main effects and interactions in the analysis of variance affected BW8 ($P<0.05$). Least-square means of BW8 for all lines as of generation 7 are presented in Fig. 16.2, and the least-squares means of BW8 for the selected lines, as deviations from the control line, are presented in Fig. 16.3. The interaction between the presence of the transgene and selection background had a significant effect on 8-week body weight means in most

Table 16.1. Means ± SE and linear contrasts for cohabitation to littering interval (CLI) and litter size (LS) in matings of non-transgenic mice (CC, NC, and NM) and oMt1a-oGH transgenic mice (TC, TM) in generation zero, pooled over two replications.

Line	N[a]	CLI (days)	LS (pups)
CC	35	22.22±0.61	12.65±0.47
NC	38	22.00±0.58	12.68±0.45
NM	33	21.53±0.63	14.22±0.49
TC	34	23.59±0.62	9.64±0.48
TM	33	23.64±0.63	11.62±0.49
Contrast (L)		L ± SE	L ± SE
T vs. N[b]		1.85±0.62**	−2.82±0.48**
S vs. C[c]		−0.21±0.62	1.76±0.48**
Interaction[d]		0.26±0.62	0.23±0.48

* $P<0.05$, ** $P<0.01$.

[a] Sample sizes.

[b] Transgenic vs. non-transgenic. Contrast value is (TM+TC−NM−NC)/2.

[c] Selected vs. control background. Contrast value is (TM−TC+NM−NC)/2.

[d] Contrast value is (TM−TC−NM+NC)/2.

generations (Table 16.2). In the control background, line TC usually had higher means for BW8 than line NC. The opposite occurred in the selected background, where line NM had higher means for BW8 than line TM in most generations.

Response to selection for increased BW8 was different from zero ($P<0.05$) in both replications of lines NM and NC. Among the transgenic lines, genetic progress was obtained in TC1, TM2 ($P<0.01$) and in TM1

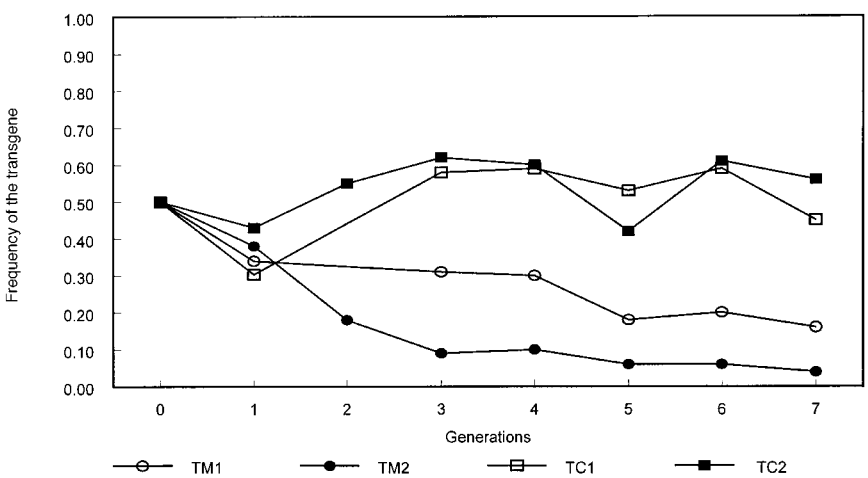

Fig. 16.1. Observed frequencies of the oMt1a-oGH transgene.

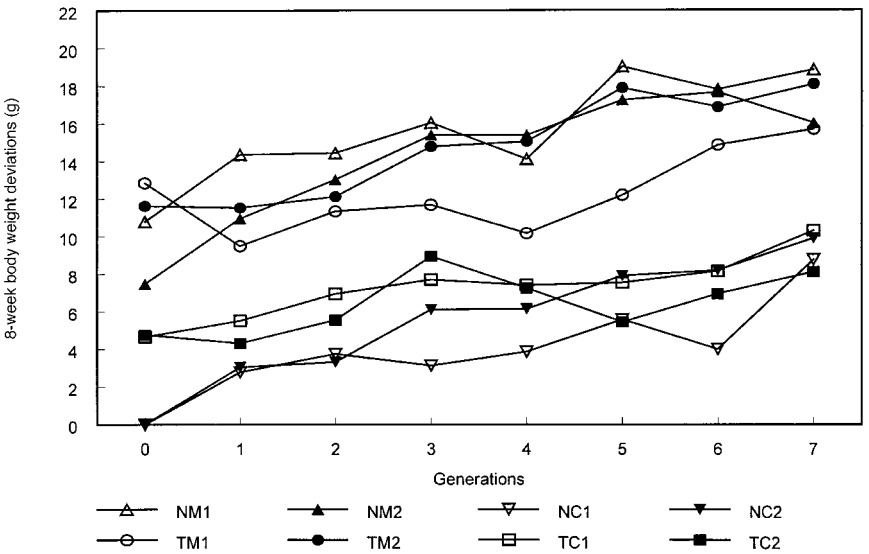

Fig. 16.2. Least-squares means ± SE for 8-week body weight as of generation 7.

(P<0.10). Estimates of genetic progress are presented in Table 16.3; realized heritabilities and the cumulative selection differentials (CSD) for each line are shown in Table 16.4. The CSD values were all around 24 g, except for line NC where the CSD was around 20 g. There was great variation in the

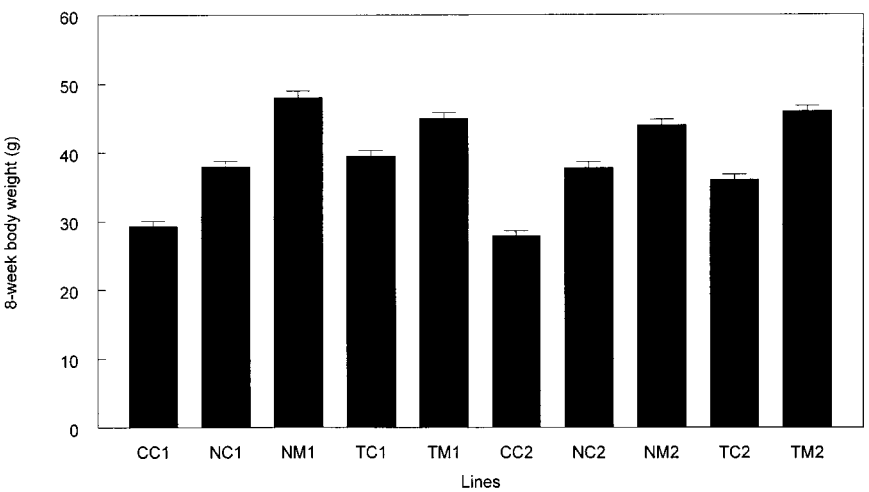

Fig. 16.3. Least-squares means for 8-week body weight, as deviations from the control lines.

Table 16.2. Orthogonal contrasts ± SE for comparisons among means of lines for 8-week body weight.

Replication	Generation	T–N[a]	M–C[b]	Interaction[c]	TM–NM	TC–NC
1	0	3.35±0.29**	9.50±0.29**	−1.29±0.29**	2.06±0.43**	4.56±0.37**
	1	−1.07±0.37**	7.79±0.37**	−3.80±0.37**	−4.88±0.54**	2.73±0.51**
	2	0.05±0.41	7.55±0.41**	−3.16±0.41**	−3.10±0.55**	3.21±0.60**
	3	0.09±0.47	8.46±0.47**	−4.47±0.47**	−4.38±0.68**	4.57±0.64**
	4	−0.20±0.32	6.51±0.32**	−3.76±0.32**	−3.96±0.45**	3.55±0.46**
	5	−2.43±0.35**	9.06±0.35**	−4.39±0.35**	−6.82±0.47**	1.96±0.52**
	6	0.61±0.39	10.26±0.39**	−3.55±0.39**	−2.94±0.53**	4.15±0.57**
	7	−0.80±0.51	7.76±0.51**	−2.35±0.51**	−3.15±0.73**	1.55±0.71*
2	0	4.47±0.29**	7.17±0.29**	−0.30±0.29	—	—
	1	0.92±0.36**	7.56±0.36**	−0.35±0.36	—	—
	2	0.66±0.38	8.14±0.38**	−1.58±0.38**	−0.92±0.56	2.24±0.50**
	3	1.12±0.38**	7.57±0.38**	−1.72±0.38**	−0.61±0.54	2.84±0.54**
	4	0.38±0.34	8.54±0.34**	−0.72±0.34*	−0.34±0.49	1.10±0.47*
	5	−0.91±0.37*	10.91±0.37**	1.55±0.37**	0.64±0.48	−2.47±0.56**
	6	−1.02±037**	9.71±0.37**	0.23±0.37	—	—
	7	−1.36±0.51**	8.06±0.57**	1.93±0.57**	2.07±0.70**	−1.79±0.74**

* $P<0.05$, ** $P<0.01$.

[a] Transgenic vs. non-transgenic. Contrast value is (TM+TC−NM−NC)/2.

[b] Selected vs. control background. Contrast value is (TM−TC+NM−NC)/2.

[c] Contrast value is (TM−TC−NM+NC)/2.

Table 16.3. Estimates of genetic progress[a] ± SE, in g per generation, in the selected lines, and linear contrasts for 8-week body weight.

Line	Replication 1	Replication 2	Pooled
NC	0.88±0.21**	1.35±0.11**	1.12±0.24[b]
NM	1.02±0.22**	1.32±0.27**	1.17±0.15
TC	0.65±0.11**	0.47±0.24	0.56±0.09
TM	0.57±0.27*	1.12±0.16**	0.85±0.28
Contrast (L)			L ± SE
T vs. N[c]			−0.44±0.20
M vs. C[d]			0.17±0.20
Interaction[e]			0.12±0.20

* $P<0.10$, ** $P<0.01$.

[a] Regressions of 8-week body weight, as deviations from the control lines, on generation number.

[b] Empirical standard errors, calculated as the standard error of replicate coefficients.

[c] Transgenic vs. non-transgenic lines. Contrast value is (TM+TC−NM−NC)/2.

[d] Selected vs. control background. Contrast value is (TM−TC+NM−NC)/2.

[e] Contrast value is (TM−TC−NM+NC)/2.

realized heritabilities across lines. The two heritability estimates within the NC line also differed greatly. Higher heritability estimates were observed in the non-transgenic lines than in the transgenic lines. Selection background did not affect heritability estimates.

Correlated responses in BW6 were observed in all selected lines except TC2, and in all lines for GAIN36 (Table 16.5). A significant correlated response in BW3 was found only in line NC2 and significant correlated responses were obtained in GAIN68 in lines NC1 and NM1. Very few significant correlated responses were observed in the fitness traits, and these were not consistent across replicates (Table 16.6). Changes in litter size were not significant in any line, except for a positive slope for litter size observed in TM2. An increase in infertility and in preweaning pup mortality over generations was observed in line NC2. Fitness index slopes were significantly negative in line NC2, and significantly positive in TM1.

Linear contrasts of slopes for body weight and weight gain traits are presented in Table 16.7. Significant contrasts were observed for BW8 in replication 2, where transgenic lines had smaller response to selection than non-transgenic lines and where lines from the selected background had a higher response to selection than lines from the control background. Three interactions were found to be significant. For BW3 in replication 2, further decomposition showed that line TC had a significantly smaller correlated response to selection than line NC, while the slopes of lines TM and NM were not different. For BW6 in replication 2 and for GAIN68 in replication

Table 16.4. Realized heritability (h^2) estimates and cumulative selection differentials (CSD), and linear contrasts for 8-week body weight, as of generation 7 of selection.

Line	Replication	Realized $h^{2\,b}$	Individual $h^{2\,b}$	CSD (g)
NC	1	0.31	0.46	19.23
	2	0.43	0.74	21.09
	Average	0.37±0.06[a]	0.60±0.14	
NM	1	0.29	0.38	23.58
	2	0.34	0.49	24.93
	Average	0.31±0.02	0.43±0.05	
TC	1	0.19	0.29	23.04
	2	0.12	0.19	22.94
	Average	0.16±0.03	0.24±0.05	
TM	1	0.17	0.26	25.55
	2	0.30	0.44	24.64
	Average	0.23±0.06	0.35±0.09	
Contrast (L)		L ± SE	L ± SE	
T vs. N[b]		−0.14±0.05	−0.22±0.09	
M vs. C[c]		0.00±0.05	−0.03±0.09	
Interaction[d]		0.07±0.05	0.14±0.09	

[a] Empirical standard errors, calculated as the standard error of replicate coefficients.
[b] Transgenic vs. non-transgenic lines. Contrast value is (TM+TC−NM−NC)/2.
[c] Selected vs. control background. Contrast value is (TM−TC+NM−NC)/2.
[d] Contrast value is (TM−TC−NM+NC)/2.

1, lines TC and NC had equivalent slopes but the average slope of the NM lines was larger than the average slope of the TM lines.

The corresponding contrasts among slopes for fitness traits are presented in Table 16.8. A significant interaction between the presence of the transgene and the selection background was found for INF, FI_1, FI_2 (both replications), and CLI (replication 1). No interaction was significant for LS and MORT. In replication 2, larger correlated responses in LS, FI_1, and FI_2, and a smaller correlated response in MORT were observed in the selected background when contrasted with the control background. Correlated responses on both fitness indexes were larger in the transgenic than in the non-transgenic lines.

Discussion

The impact of the introduction of foreign DNA into commercial livestock populations will be felt when production or disease resistance can be raised to higher levels. Since selection in transgenic populations will be done on

Table 16.5. Regression coefficients ± SE of correlated responses in body weight and weight gain traits, deviated from control lines, in the selected lines.

Line	Replication	BW3[a] (g)	BW6 (g)	GAIN36 (g)	GAIN68 (g)
NC	1	0.09±0.12[b]	0.69±0.20***	0.60±0.11***	0.18±0.04***
	2	0.46±0.10***	1.13±0.12***	0.66±0.13***	0.16±0.08
NM	1	−0.14±0.12	0.50±0.22*	0.67±0.11***	0.46±0.06***
	2	0.13±0.09	0.96±0.24***	0.86±0.23**	0.27±0.14
TC	1	0.18±0.11	0.54±0.13***	0.38±0.19*	0.09±0.11
	2	0.06±0.14	0.41±0.25	0.48±0.14**	0.01±0.14
TM	1	0.15±0.21	0.74±0.19***	0.60±0.14***	−0.11±0.17
	2	0.17±0.11	1.03±0.14***	0.86±0.17***	0.03±0.19

*P<0.10, **P<0.05, ***P<0.01.

[a] BW3, 3-week body weight; BW6, 6-week body weight; GAIN36, body weight gain from 3 to 6 weeks; GAIN68, body weight gain from 6 to 8 weeks.

[b] Standard error calculated from least squares regression.

nucleus or elite herds, the integration of a transgene must be compatible with overall selection goals. The maintenance of transgenic populations will depend on the economic value of the transgene and also on whether further selection causes a reduction in transgene frequency because of relatively poor viability of embryos or reproduction in adults. Regulated expression and stable integration of the transgene insert and transmission to offspring are critical in the development of useful transgenic livestock (Sabour *et al.*, 1991).

Reduction in the frequency of the transgene in both replications of the TM line and the lower response to selection in TM compared with NM may be explained by the fact that past selection may have increased the frequencies of other alleles involved in the production of growth hormone or may have affected genes downstream in the growth hormone cascade. Evidence favouring this interpretation is the negligible additive effect of the transgene for 8-week body weight in TM males and the smaller additive effect in TM compared with TC females (Siewerdt *et al.*, 1998). This factor would leave less opportunity for contribution to growth enhancement due to the expression of the transgene. A diametrically opposite explanation is that past selection for rapid 3- to 6-week body weight gain may have actually caused the reduction of the average level of circulating growth hormone, as observed by Medrano *et al.* (1991). The increase in circulating growth hormone induced by the transgene could be upsetting the physiological balance in the mice of the selected background. Another factor that may be partly responsible for the lower response to selection in TM compared with NM is a reduced fitness of transgenic embryos. The reduction in the frequency of the transgene is partially consistent with the results that Sabour *et al.* (1991) obtained in lines of mice carrying the rat growth hormone transgene. However, these authors found that the

Table 16.6. Regression coefficients ± SE of correlated responses in fitness traits, deviated from control lines, in the selected lines.

Line	Replication	INF (%)[a]	CLI (d)	LS (pups)	MORT (%)	FI_1	FI_2
NC	1	−0.62±1.42[b]	0.08±0.19	0.01±0.16	−0.56±0.89	0.16±0.19	0.11±0.17
	2	4.56±1.42***	0.17±0.19	0.06±0.16	1.99±0.89**	−0.73±0.19***	−0.62±0.17***
NM	1	−0.20±1.42	0.44±0.19	−0.04±0.16	−0.12±0.89	−0.01±0.19	−0.10±0.17
	2	0.98±1.42	0.24±0.19	0.23±0.16	0.19±0.89	0.03±0.19	−0.02±0.17
TC	1	2.23±1.42	0.18±0.19	−0.12±0.16	−1.22±0.89	−0.20±0.19	−0.19±0.17
	2	0.62±1.42	−0.19±0.19	0.09±0.16	1.81±0.89	−0.15±0.19	−0.08±0.17
TM	1	−2.46±1.42*	−0.32±0.19*	0.20±0.16	−1.32±0.89	0.57±0.19***	0.52±0.17***
	2	1.93±1.42	0.08±0.19	0.52±0.16***	0.89±0.89	0.09±0.19	0.06±0.17

*P<0.10, **P<0.05, ***P<0.01.

[a] INF, infertile matings; CLI, cohabitation to littering interval; LS, litter size; MORT, preweaning pup mortality; FI_1, fitness index 1; FI_2, fitness index 2. See text for definition of fitness indexes.

[b] Standard error calculated from least squares regression.

Table 16.7. Linear contrasts of slopes for body weight and weight gain traits (generations 0–7).

Contrast	Replication	BW3[a] (g)	BW6 (g)	BW8 (g)	GAIN36 (g)	GAIN68 (g)
T vs. N[b]	1	0.19±0.15[e]	0.05±0.19	−0.41±0.26	−0.15±0.14	−0.33±0.11***
	2	−0.18±0.11	−0.33±0.20	−0.74±0.21***	−0.09±0.17	−0.20±0.14
M vs. C[c]	1	−0.13±0.15	0.01±0.19	0.11±0.26	0.15±0.14	0.04±0.11
	2	−0.11±0.11	0.23±0.20	0.54±0.21**	0.29±0.17	0.07±0.14
Interaction[d]	1	0.10±0.15	0.20±0.19	−0.31±0.26	0.08±0.14	−0.24±0.11**
	2	0.22±0.11*	0.40±0.20*	0.27±0.21	0.09±0.17	−0.05±0.14
TM–NM	1	—	—	—	—	−0.57±0.13***
	2	0.04±0.10	−0.72±0.20***	—	—	—
TC–NC	1	—	—	—	—	−0.09±0.08
	2	−0.40±0.12***	0.07±0.20	—		——

*P<0.10, **P<0.05, ***P<0.01.

[a] See Table 16.5, footnote [a], for definition of traits.

[b] Transgenic vs. non-transgenic lines. Contrast value is (TM+TC−NM−NC)/2.

[c] Selected vs. control background. Contrast value is (TM−TC+NM−NC)/2.

[d] Contrast value is (TM−TC−NM+NC)/2.

[e] Standard error calculated from least squares regression.

Table 16.8. Linear contrasts of slopes for reproductive traits (generations 0–7).

Contrast	Replication	INF (%)[a]	CLI (d)	LS (pups)	MORT (%)	FI_1	FI_2
T vs. N[b]	1	0.29±1.15[e]	−0.33±0.14**	0.05±0.10	−0.92±0.69	0.11±0.16	0.16±0.14
	2	−1.50±1.15	−0.25±0.14*	0.16±0.10	0.26±0.69	0.32±0.16*	0.31±0.14**
M vs. C[c]	1	−2.13±1.15*	−0.07±0.14	0.13±0.12	0.17±0.57	0.30±0.17*	0.26±0.14*
	2	−1.14±1.15	0.17±0.14	0.31±0.12***	−1.36±0.57**	0.50±0.17***	0.37±0.14**
Interaction[d]	1	−2.55±1.25*	−0.43±0.12***	0.18±0.13	−0.27±0.88	0.47±0.13***	0.46±0.10***
	2	−2.45±1.25*	0.10±0.12	0.13±0.13	0.44±0.88	−0.26±0.13**	−0.23±0.10**

*P<0.10, **P<0.05, ***P<0.01.

[a] See Table 16.6, footnote [a] for definitions of traits.

[b] Transgenic vs. non-transgenic lines. Contrast value is (TM+TC−NM−NC)/2.

[c] Selected vs. control background. Contrast value is (TM−TC+NM−NC)/2.

[d] Contrast value is (TM−TC−NM+NC)/2.

[e] Standard error calculated from least squares regression.

reduction in the frequency of the rat growth hormone (rGH) transgene was independent of selection background, which disagrees with the results reported here. The different results can be explained by noting that females transgenic for the rGH have this growth hormone transgene chronically expressed, and they have a severe reduction in reproductive performance leading to a rapid loss of the transgene from the population.

Because of the large additive genetic effect of the oMT1a-oGH transgene that contributes to the total phenotypic value of 8-week body weight in both males and females in the unselected background (Siewerdt *et al.*, 1998), upward selection for this trait in the presence of the transgene at a starting frequency of 0.5 was expected to result in rapid genetic progress and an increase in the transgene frequency approaching one after seven generations. However, the selection response was actually higher in the unselected background lines without the transgene than in those with the transgene, and the frequency of the transgene only increased marginally in the TC replicates. A possible explanation for this finding is that embryos carrying the transgene construct have reduced viability (Eisen *et al.*, 1995; Clutter *et al.*, 1996; Siewerdt *et al.*, 1998).

In generation 0, there were no significant differences in pup mortality during the preweaning period between transgenic and non-transgenic lines (data not shown). The absence of line differences in preweaning pup mortality between non-transgenic and hemizygous transgenic crosses suggests that higher zygotic loss and prenatal and perinatal mortality in homozygous transgenic and/or hemizygous transgenic progeny genotypes may explain the lower litter size in hemizygous transgenic crosses. Perinatal mortality differences cannot be excluded entirely because litter size was recorded at 1 day of age, so any pups that may have died on the day of birth and were eaten by the mother before data were recorded would be included in the prenatal group. The perinatal period probably contributes minimally, however, since dams were checked daily for litter births and dead pups were routinely counted and removed.

An explanation for the reduced litter size in the hemizygous transgenic crosses is the reduced reproductive performance of transgenic females which could occur as a consequence of overexpression of the transgene (Bartke *et al.*, 1994). However, the transgenic female parents of generation 0 did not receive $ZnSO_4$ in the drinking water at any time so the transgene should not have been chronically expressed. Nevertheless, some leakage of the oMt1a-oGH transgene is known to occur (J.D. Murray, personal communication), which could have a negative effect on litter size. The 2-week withdrawal period could offset part of the negative effects of leakage. This is unlikely, however, because in cases where transgenic females are mated 2 weeks after the $ZnSO_4$ is withdrawn, litter size is actually enhanced due to an increase in ovulation rate (Murray and Pomp, 1995; Eisen *et al.*, 1995).

Fitness models affecting prenatal survival that may explain the difference in litter size between hemizygous transgenic matings and non-transgenic

matings within the same genetic background are given in Table 16.9. The absolute and proportional reduction in litter size of the hemizygous transgenic crosses was larger in the control than in the high-growth background (3.04 pups versus 2.60 pups; 24.1% and 18.3%, respectively). Although this difference was not significant, the models having a reduced fitness for the transgene lowered the expected number of transgenic progeny more in the control background than in the selected background.

The fitness models assume that the transgene segregates in a Mendelian fashion with respective fitness values for T/T, T/−, and −/− of W_1, W_2, and W_3. The fitness models are: (1) equal fitness for all genotypes; (2) selection against T/T, $W_1<W_2=W_3$; (3) equal selection against T/T and T/−, $W_1=W_2<W_3$; (4) selection against T/T which is greater than the selection against T/−, $W_1<W_2<W_3$; (5) selection against T/− which is greater than the selection against T/T, $W_1>W_2<W_3$ (Table 16.9). Model 1 is based on the expectation of progeny genotypes in the absence of differential viability for the transgene. In model 4, the relative fitness values were calculated based on earlier results from backcross data, which showed an observed ratio of 0.45 hemizygous transgenics to 0.55 non-transgenics in the progeny, yielding relative fitness coefficients of 0.82–1.00. The relative fitness coefficient for the homozygous transgenic pups was then calculated by difference, based on the observed litter size (Table 16.1). Assuming an initial gene frequency of 0.5 for the transgene, the expected gene frequencies in the next generation were 0.342, 0.447, 0.401 and 0.483 for models 2, 3, 4 and 5, respectively, in the randomly selected background compared with the observed frequency of 0.321. In the high growth background, the expected gene frequencies were 0.388, 0.463, 0.443 and 0.485 for models 2, 3, 4 and 5, respectively, compared with the observed frequency of 0.296. There would appear to be prenatal zygotic or embryonic selection against the transgene, but the selection may be greater than predicted from the simple models presented.

A fitness handicap associated with the transgene would cause a reduction in the frequency of the transgene in the long run. For instance, in line TC where the effect of the transgene on BW8 is large (Siewerdt *et al.*, 1998), the relative contribution of other loci is smaller. If transgenic individuals are less fit than non-transgenics, then the frequency of the transgene should decrease slightly from one generation to another. If the population is under selection, there will be room to obtain improvement in other loci affecting BW8. Because frequencies of favourable alleles will increase at these loci, the relative contribution of the transgene to the genetic variation should be expected to steadily decline, increasing the chance of obtaining even further genetic progress on those other loci in future generations. Thus, the frequency of the transgene should be reduced in the long run. However, this does not imply that one should expect that every transgene would be eliminated as a consequence of selection in the long run. If the relative effect of a transgene is large enough to overcome

Table 16.9. Prenatal viability in fitness models that could explain the difference in litter size between mating of hemizygous transgenic mice versus matings of non-transgenic mice in generation 0.

	Unselected background							High-growth selected background						
	Relative fitness			Pup distribution				Relative fitness			Pup distribution			
Model[a]	T/T	T/−	−/−	T/T	T/−	−/−	Litter size (pups)	T/T	T/−	−/−	T/T	T/−	−/−	Litter size (pups)
1. $W_1=W_2=W_3$	1	1	1	3.17	6.34	3.17	12.68[b]	1	1	1	3.555	7.11	3.555	14.22[d]
2. $W_1<W_2=W_3$	0.04	1	1	0.13	6.34	3.17	9.64[c]	0.27	1	1	0.95	7.11	3.56	11.62[e]
3. $W_1=W_2<W_3$	0.68	0.68	1	2.16	4.31	3.17	9.64[c]	0.76	0.76	1	2.69	5.37	3.56	11.62[e]
4. $W_1<W_2<W_3$	0.40	0.82	1	1.27	5.20	3.17	9.64[c]	0.63	0.82	1	2.23	5.83	3.56	11.62[e]
5. $W_1>W_2<W_3$	0.90	0.57	1	2.85	3.61	3.17	9.64[c]	0.90	0.68	1	3.20	4.86	3.56	11.62[e]

[a] W_1, W_2, W_3 are relative fitness values for homozygous transgenics (T/T), hemizygous transgenics (T/−), and non-transgenics (−/−).
[b] Mean litter size for the NM line in generation 0.
[c] Mean litter size for the TM line in generation 0.
[d] Mean litter size for the NC line in generation 0.
[e] Mean litter size for the TC line in generation 0.

the contribution of all other loci involved in a specific phenotypic expression, then its frequency should only decay because of reduced fitness of the carriers. Whether it is a fitness handicap or a situation where the contribution of the transgene was diminished because of previous selection, the correct explanation for the reduction in the frequency of the transgene on lines TM1 and TM2 is a question left to be answered by future research.

No slopes for the fitness indices were significantly negative in the transgenic lines, and in line TM1 both slopes were even positive (Table 16.6). Also, when contrasted with non-transgenic lines, the slopes of the transgenic lines were significantly larger in replication 2 (Table 16.8). These fitness indexes, however, did not consider prenatal embryonic or fetal survival.

For BW8, the interaction between the effects of the transgene and the selection background was significant in most of the generations, as shown in Table 16.2. An identified pattern is that TC mice are larger than NC, but TM mice are smaller than NM. Results suggest that the oMt1a-oGH transgene has a greater effect when incorporated into populations with no past selection for increased body weight. Favourable correlated responses observed on BW6 and GAIN36 suggest pleiotropic effects associated with the transgene insert that can be advantageously exploited. Polygenic frequencies that affect the correlated traits may have also changed through indirect selection, but apparently the experimental design used does not allow isolation of the effects of polygenic inheritance from pleiotropic effects associated with the transgene.

The presence of an activated oMt1a-oGH transgene in lines TM and TC led to smaller realized heritability estimates when compared with the lines NM and NC, despite creating a larger phenotypic variance which resulted in higher selection differentials. In agreement with these results, Clutter *et al.* (1996) also found heritability estimates for BW8 to be larger in lines without the oMt1a-oGH transgene than in transgenic lines. The introduction of a transgene with large effect on a trait is expected to increase the heritability of the trait unless the transgene shows overdominance and is at or near the equilibrium gene frequency or the transgene introduced a large epistatic component. Although the oMt1a-oGH transgene exhibits overdominance effects (Siewerdt *et al.*, 1998), the equilibrium frequency of the transgene is about 0.9, which is much higher than the frequency in the present lines. Therefore the likely explanation for the reduced heritability is epistasis. In addition, environmental variation may have been increased due to a possible reduced buffering capacity of the transgenic mice. In line TC, genetic progress was obtained both in the form of a slight increase in the frequency of the transgene and in the accumulation of favourable alleles at other loci. In line TM the genetic progress was chiefly obtained at other loci that affect BW8, since the frequency of the transgene was reduced from the initial value of 0.5. This finding showed that there was still opportunity for genetic improvement in the crosses involving a line heavily selected in the

past for growth. From the results reported here it is suggested that in choosing a transgene to be incorporated into commercial stocks one should aim at a construct which would affect traits not previously subjected to intense selection, since the effect of the transgene may not have as much impact as in traits which had received little selection pressure previously or may interact unfavourably with the artificially selected genotypes.

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