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Decreased frequency of the rat growth hormone transgene in mouse populations with or without selection for increased adult body weight *

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Summary. Frequencies of mice with the rat growth hormone (*rGH*) transgene were examined in lines derived from two genetic bases (P/W and P/C). The genetic bases were developed from males (P) with the *rGH* transgene, mated with non-transgenic females of different origin: a line previously selected for large body size (W) and a corresponding unselected control line (C). They were maintained for six generations under random mating with or without selection for increased 42-day body weight. The frequencies of P/W and P/C males with the *rGH* transgene wer 0.075 and 0.300, respectively at generation 0 of the genetic bases. They were significantly ($P < 0.05$) lower than the expected frequency (about 0.5). At generation 6, the frequencies had decreased further both in selected and unselected lines (ranging from 0.025 to 0.125). Decreased frequencies of mice with the transgene were confirmed in a separate experiment testing segregation of the transgene. The reasons for these decreases are not clear. The results suggest that transgenes need to be monitored when transgenic animals are mated with animals of different origin.

Key words: Transgene – Mice – Growth – Reproduction – Selection response

Introduction

Although various strategies for producing transgenic animals have been employed (reviewed by Palmiter and Brinster 1986; Jaenisch 1988), direct microinjection of purified cloned DNA into the egg pronucleus has been the most reliable and succesful method of gene transfer. With this approach, mice with increased growth, expressing exogenous growth hormone or growth-releasing factor genes, have been produced (Palmiter et al. 1982a; 1983). Similar attempts have been made to produce transgenic livestock and the results are very promising (Hammer et al. 1985a; Pursel et al. 1989).

The number of transgenic animals produced from microinjection procedures is usually limited. To increase the number of transgenic individuals, they must be mated with either transgenic or non transgenic animals. Stable transmission of the transgene(s) is critical to build up a population containing the transgenic animals. Transgenes are known to be transmitted in a Mendelian manner when transgenic mice are mated with non transgenic mice of the same origin (Constantini and Lacy 1981; Gordon and Ruddle 1981; Wagner et al. 1981). The outcome of mating transgenic mice with non transgenic of a different origin has not been investigated.

The main objective of the present study was to examine frequencies of mice with the rat growth hormone (*rGH*) gene in lines of mice derived from two different genetic bases. We report a significant decrease in the frequency of mice with the *rGH* gene after six generations of random mating.

Materials and methods

Mice

Five male transgenic mice (P) carrying the mouse metallothionein – rat growth hormone transgene (MT-*rGH*) and five males without the transgene (N) were obtained from the University of Pennsylvania (the generous gift of Dr. R.L. Brinster). The P mice, registered officially as Tg (Mt-1, GH) Bri2, originated from a male (MGH-10, Palmiter et al. 1982a) that was obtained from microinjected eggs (C57BL/6 × SJL). The P mice were hemizygous for the MT-*rGH* and each contained eight copies of

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the transgene integrated at a single chromosomal location (Dr. R. Behringer, University of Pennsylvania, personal communication).

At the Animal Research Centre, the five P and five N males were mated with females of two lines (W and C) at the ratio of one male to three females. Line W was selected at the Center for increased 42-day body weight; line C was an unselected control line. They both originated from a population synthesized from C3H/He, C57BL/6J, CBA/J, and SWR/J inbred strains (Nagai and Kristjansson 1970). Selection was conducted for 15 generations (Nagai et al. 1978). Subsequently, each line was maintained by random mating of 25 pairs every generation until generation 82.

Females of the W and C lines at generation 82 were mated, 2 weeks apart, at 9 weeks of age with P or N males to produce F_1 progeny (Fig. 1). The resulting female progeny (F_1) were backcrossed to the original P or N males at 7 weeks of age to produce backcross progeny B_1. F_1 mice in second litters were mated randomly to produce F_2 progenies. Forty males and females consisting of equal numbers of F_2 and B_1 mice were randomly pair-mated to produce two genetic bases: P/W, P/C. In each base, 40 pairs were randomly mated for two generations. The progeny of the second random mating was designated generation 0 (Fig. 1), in which each litter was divided in half to derive a line to be selected for increased 42-day weight and an unselected control line. Each of the four lines contained 42-day weight and an unselected control line. Each of the four lines contained 60 pairs after generation 2. Male breeders at generation 0 and 6 were examined for the *rGH* transgene. In a separate experiment testing segregation of the transgene, four males at generation 6 (two from the P/C selected and two from the P/C unselected lines) that carried the *rGH* transgene were mated with eight females from a base line (N/C) without the transgene. The resulting adult progeny (42 days old) were examined by slot blot hybridization for the transgene, to test segregation based on an expected 1:1 ratio.

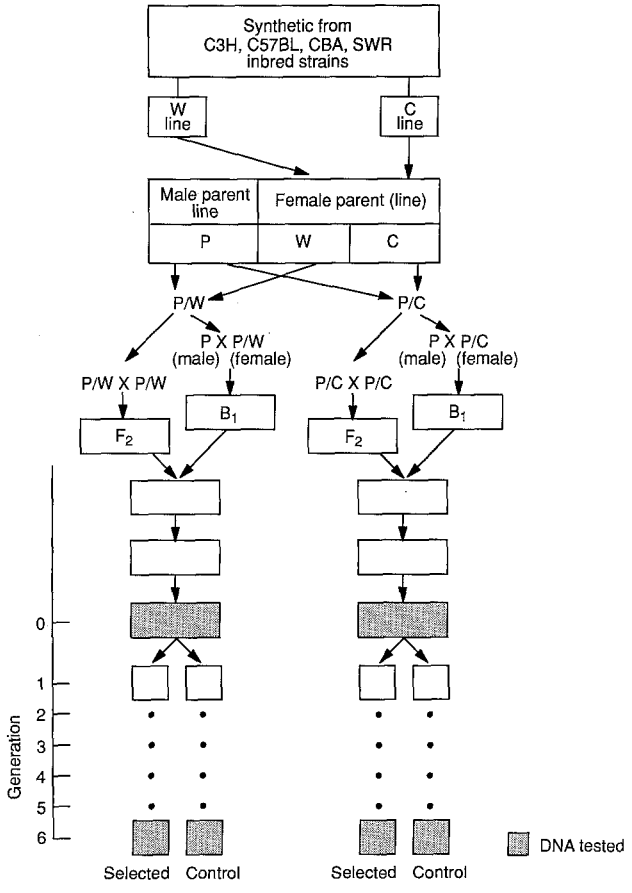

Fig. 1. Breeding history of lines used. For notation, see "Materials and methods"

Body weight at 42 days was recorded in both selected and unselected lines. Throughout the experiment, males cohabited with females for 14 days. At parturition, litter size was not standardized until generation 0 and, thereafter, was standardized to eight at birth. The eight young were chosen at random. Individual progeny were sexed at weaning (21 days). Each cage ($29.2 \times 12.7 \times 19.1$ cm) contained mice of the same sex after weaning. Mice were maintained in a facility where temperatures ranged from 21 to 24 °C and relative humidity from 50 to 70%. Pelleted feed and water, without added metal ingredients (e.g., zinc), were supplied ad libitum.

DNA isolation

High-molecular-weight DNA was isolated from 1–2 cm of mouse tails as described by Hogan et al. (1986), dissolved in TE buffer (10 m*M* Tris, pH 7.4, 1 m*M* EDTA), and the DNA amount was measured by fluorometry (Labarca and Paigen 1980).

Analysis of DNA

Slot blot hybridization was performed as described by Brinster et al. (1985). For analysis, DNA (5 µg) was transferred to a pre cut nitrocellulose membrane in BRL Hybri-slot blot apparatus as per Davis et al. (1986). Twenty samples were loaded on each membrane. The *rGH* fragment (1 ng) was transferred as a positive control.

Probe preparation

The plasmid p*rGH*-1, kindly provided by Dr. R.D. Palmiter (University of Washington), was then transferred to *Escherichia coli* strain JM/109, using the procedure of Cohen et al. (1972). It contains a 350-bp XhoI-PvuII fragment that includes most of the first exon of the *rGH* gene and part of the first intron, cloned into the vector pGEM-2 (see Promega Bio/Can Scientific, Inc., Technical Manual). Plasmid DNA was digested with EcoRI and HindIII (2 units/µg DNA) at 37 °C using 1 × TA buffer according to the manufacturer's (Boehringer Mannheim) procedure. After separation of restriction fragments on a 0.8% agarose gel in TBE, a 400-bp DNA fragment, corresponding to *rGH*, was excised and isolated using an IBI electroeluter. The purified *rGH* fragment was nick-translated using the kit from Boehringer Mannheim and 3,000 Ci/mmol [α^{32}P]dCTP (Amersham, Canada) to a specific activity of $1-3 \times 10^8$ cpm per µg DNA.

Hybridization

The nitrocellulose filters were pre hybridized for 1 h at 50 °C in the following solution: 50% formamide, 6 × SSC, 1% SDS, 0.1% Tween 20, and 100 µg/ml tRNA. Hybridization was carried out overnight at 50 °C in fresh prehybridization buffer with the addition of 100 ng of the heat-denatured ^{32}P-labelled *rGH* probe. After hybridization, the filters were washed once in 1 × SSC and 0.1% SDS, for 30 min at room temperature (low stringency). The filters were blotted on Whatman paper and autoradiographed overnight at −70 °C using Kodak XAR-2 film and DuPont Cronex intensifying screens. The filters were then washed once in 0.1 × SSC and 0.1% SDS for 30 min at 65 °C (high stringency) and set up for a second overnight expo-

Table 1. Frequency of male mice with the rat growth hormone transgene in two different genetic bases

Genetic base	Line	Generation			
		0		6	
		Number of positive males	Frequency	Number of positive males	Frequency
P/W	Selected	3[a]	0.075±0.042*	2	0.050±0.034
	Unselected control			2	0.050±0.034
P/C	Selected	12	0.30±0.072	1	0.025±0.024
	Unselected control			5	0.125±0.052

[a] Out of 40 males sampled at random
* Standard error

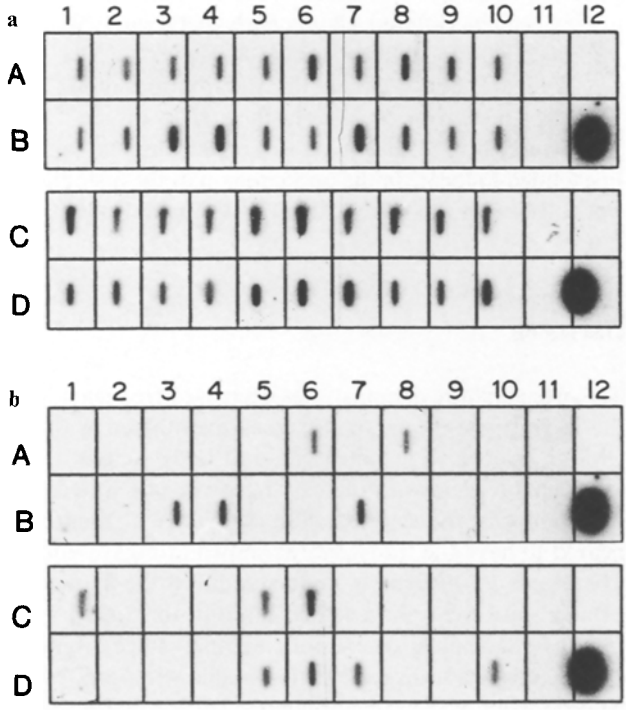

Fig. 2a and b. Slot blot hybridization of DNA from mice populations carrying *rGH* gene. Five micrograms DNA were spotted onto nitrocellulose, baked, and hybridized with Eco RI-HindIII probe as described in the experimental procedures. *a*: membrane filter washed under low-stringency conditions. *b*: same membrane, as shown above, washed under high-stringency conditions. Plasmid DNA carrying *rGH* DNA fragment (1 ng) was blotted in the last slot

sure as described above. Sequence homologies between mouse and rat growth hormone genes allowed hybridization of the *rGH* transgene probe with mouse DNA during the low-stringency wash (Fig. 2), and thus verify the presence of similar amounts of DNA in each slot of the membrane. However, when the same membranes were washed at high stringency, the *rGH* probe remained hybridized only to DNA from mice carrying the *rGH* transgene (Fig. 2).

Statistical procedures

Analyses of variance were conducted in each genetic base (line) to compare the 42-day body weight between two groups of mice with and without the *rGH* transgene. Chi-square tests were used to compare frequencies of mice with the *rGH* transgene between generations 0 and 6 within line, and for segregation of the transgene in a separate experiment.

Results

Figure 2 shows the results of a typical slot hybridization that identifies mice with or without the *rGH* transgene. The intensity of the autoradiographic signal was similar in all the positive samples tested, suggesting that *rGH* transgene copy number were similar.

Table 1 shows frequencies of mice with the *rGH* transgene. The frequencies at generation 0 were significantly ($P<0.05$) lower than the frequency of 0.5 expected from simple Mendelian segregations. A nonsignificant ($P\geq 0.05$) decrease in frequencies was observed at generation 6 for the unselected control P/W and P/C lines. However, after selection for 42-day body weight in each of the selected P/W and P/C lines, there was a significant decrease in the P/C line only for the *rGH* frequency ($P<0.05$). There was a significant difference ($P<0.05$) in transgene frequency between P/W and P/C lines at generation 0.

In a separate experiment, four males tested positive for *rGH*, mated with eight females, produced a total of 63 progeny (31 adult males, 29 adult females, and 3 stillbirths). Slot blot hybridization tests revealed that each litter contained at least 3 progeny negative for the transgene, indicating that the males were hemizygous for the transgene. Of 60 adult progeny examined, 21 progeny were positive, while 39 progeny were negative. A χ^2 test based on a 1:1 ratio showed that the deviation from the expected ratio was significant ($P<0.05$), but the heterogeneity among litters was not (Table 2). It appeared that the results were not affected by the sex of the progeny (10

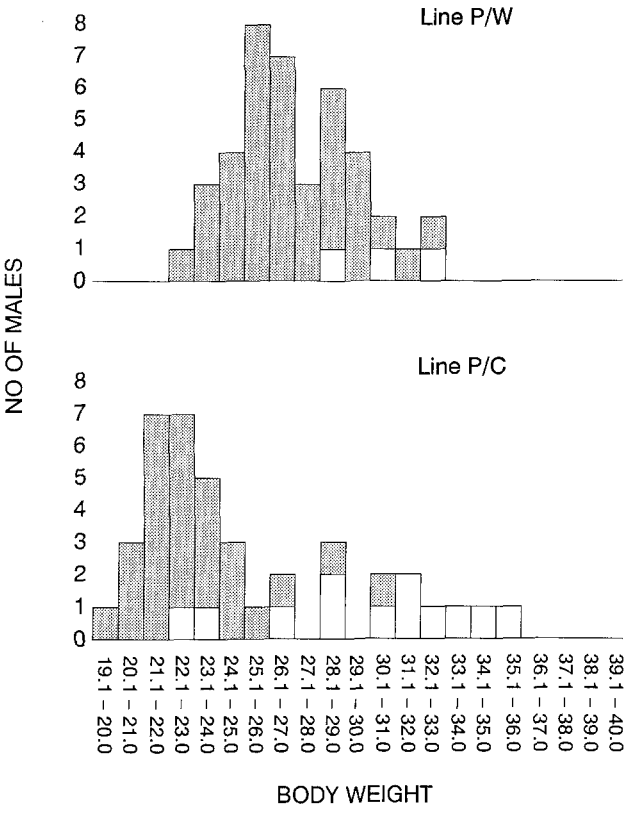

Fig. 3. Distribution of 42-day body weight (g) of male mice in two lines, P/W and P/C, at generation 0. *Open squares* (□) represent mice with the *rGH* transgene

Table 2. Test of segregation of the *rGH* transgene based on a 1:1 ratio in the progeny

Source	χ^2	*df*	Significance (*P*)
Pooled	5.40	1	0.02*
Heterogeneity	3.51	7	0.80
Total	8.91	8	

positive males and 20 negative males, and 11 positive females and 19 negative females). Of the three still births, two were positive and one was negative.

In general, mice with the *rGH* transgene had heavier 42-day body weight (Table 3) than those without. Analysis of variance of 42-day weight revealed that the difference between two groups of mice, positive and negative for the *rGH* transgene, was highly significant ($P<0.01$) in the P/W and P/C genetic bases at generation 0, and in the P/C selected and unselected lines at generation 6 (Table 3). Neither selected nor control lines of the P/W genetic base, at generation 6, differed significantly in mean 42-day weight between positive and negative groups. No significant difference in mean 42-day weight was observed between P/W and P/C mice with the transgene at generation 0 (30.2 g versus 30.0 g), while mean 42-day weight differed significantly between P/W and P/C mice without the transgene (26.9 g versus 23.1 g).

Figures 3 and 4 show the distribution of 42-day weight of mice with or without the *rGH* transgene. It is interesting to note that mice with the transgene in P/C line tended to locate in the upper region of the histogram, but those with the transgene in P/W line did not stand out.

Discussion

The present study demonstrates decreased frequencies of the *rGH* transgene in mouse lines maintained with or without selection for increased adult body weight.

Male breeders with increased growth rate were used for mating in the selected P/C line. Most of them appeared to have the transgene, as shown in Figs. 3 and 4. Therefore, it is difficult to understand how the frequency of mice with the transgene decreased in this line.

At Philadelphia, where our transgenic mice originated, hemizygous transgenic males, mated with non transgenic females of the same origin (C57BL/6 × SJL), trans-

Table 3. Mean 42-day body weight of male mice with or without the *rGH* transgene

Genetic base	Generation	Line	*rGH* positive		*rGH* negative		
			No.	g	No.	g	
P/C	0		12	30.0±1.2[a]	28	23.1±0.5	**[b]
	6	Selected	1	36.4±–	39	28.4±0.3	**
	6	Unselected	5	34.0±0.8	35	25.4±0.3	**
P/W	0		3	30.2±1.4	37	26.9±0.4	**
	6	Selected	2	32.3±2.1	38	32.0±0.4	NS
	6	Unselected	2	31.2±0.9	38	28.9±0.5	NS

[a] Mean body weight ± standard error
[b] ** Significant difference between positive and negative groups ($P<0.01$);
NS, not significant ($P>0.05$)

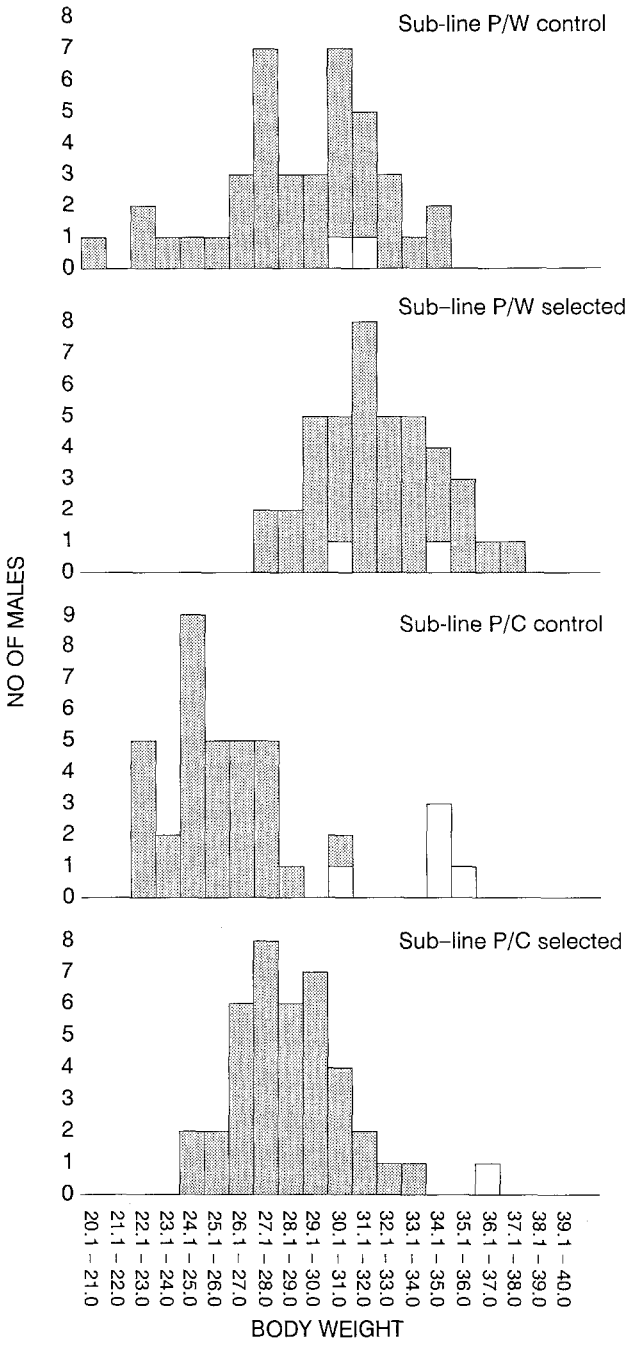

Fig. 4. Distribution of 42-day body weight (g) of male mice after six generations of random mating with or without selection for increased 42-day body weight. *Open squares* (□) represent mice with the *rGH* transgene. The histogram clearly shows that mice with the *rGH* transgene are located in the upper region in the P/C line. This is not the case in the P/W line

mitted the eight copies of the *rGH* transgene to about half of their offspring, indicating stable integration of the *rGH* transgene into a single chromosomal location and normal Mendelian transmission (Palmiter et al. 1982a). In Ottawa, transgenic males were mated with females of different origins. Throughout the Ottawa experiment from generation 0 to 6, fertility ranged mostly from 85% to 100%. Thus, it is unlikely that infertility of females contributed to the decreased frequency observed in the present study. Such low fertility was previously observed in females with growth hormone transgene (*rGH, bGH,* or *hGH*) (Hammer et al. 1984, 1985b).

Genomic instability due to the deletion of some gene copies from a large tandem array has been reported (Palmiter et al. 1982b). In the present study, however, the intensity of the hybridization signal of all males testing positive for the transgene was similar. It is therefore unlikely that the gene copy number was significantly altered.

In *Drosophila* (Hartl and Hiraizumi 1976) and mice (Bennett 1975), genetic factors have been observed that lead to non random segregation of specific loci and cause differential survival of meiotic products to favor the mutant. In the present study, the survival of non-mutant products seems to be favoured. Such results would be expected if the inserted genetic material acted to reduce the transmission of a gene necessary for the formation of viable gametes either directly (via insertion into an essential gene) or indirectly (via insertion into a modifier gene). An insertional mutation that directly affected the formation of fertile spermatozoa has been described in transgenic mice (Palmiter et al. 1984). However, since normal transmission of the *rGH* was observed in the C57BL/6 × SJL genetic background, a similar direct effect is unlikely here. Variable expression of the transgene in offspring due to the effects of segregating modifier genes or sequences has been suggested previously (Palmiter et al. 1982b).

This type of indirectly modulated gene expression is consistent with the present observation in that the decrease in the frequency of mice with the transgene at generation 0 seemed to be indepent on the lines of mice (W or C) mated to the original transgenic males. The exact underlying cause of this decreased frequency, however, remains to be determined.

While the regulated expression of the transferred gene is of fundamental importance in the development of any useful transgenic livestock, stable integration of the transgene and its transmission to offspring are both critical. This work emphasizes the importance of careful evaluation of the stability of transgene transmission to offspring for several generations, particularly when founder transgenic parents are mated with animals of different genetic origin.

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