DEVELOPMENT OF LINES OF MICE WITH OR WITHOUT RAT GROWTH HORMONE TRANSGENE AND ITS FREQUENCY IN MICE MAINTAINED UNDER RANDOM MATING

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SUMMARY

Male mice with (P) or without (N) rat growth hormone transgene were mated with females of two lines (W and C) to produce foundation stocks of four lines for selection to increase adult body weight. Frequencies of mice with the transgene were monitored in selected and control lines at generations 0 and 6. The F_1 progeny of sires with the transgene compared to the F_1 progeny of sires without the transgene were 16% heavier ($P<0.01$) in 42-day weight in both dam lines, indicating that the transgene was transmitted to the progeny. Percentages of males with the transgene at generations 0 and 6 were lower than expected. Thus, the frequency of the transgene introduced to conventional mice was not maintained in these lines of mice under random mating with or without selection for increased adult body weight.

INTRODUCTION

Progress in recombinant DNA and embryo manipulation techniques led to production of many transgenic mouse stocks. They are useful for studying gene expression, tissue specificity and other phenomena. One such transgenic mouse stock has a transgene containing the promoter of the mouse metallothionein-I gene fused to the structural gene of the rat growth hormone (MT-rGH). The mice were reported to exhibit rapid growth rate and large body size with high levels of the transgene mRNA in their liver and growth hormone in their serum (Palmiter et al. 1982a). The rat growth hormone transgene was transmitted to progeny as a simple Mendelian trait (Hammer et al. 1984).

Since production of transgenic animals requires high skills and elaborate equipment, the number of transgenic animals produced is usually small. Under normal circumstances, useful transgenic animals need to be propagated in order to be thoroughly tested before their use in animal agriculture. General principles of mating systems that include monitoring of the transgene and selection of superior animals have not been studied. The establishment of such a system is necessary because production of potentially useful transgenic livestock can be expected in the near future.

Mice are a useful pilot organism for studying principles of animal breeding. The principles transcend species, maintenance cost of mice is small, and their generation interval and lifespan are short. This is a preliminary report from a study that had the objectives to 1) examine the growth of crossbred progeny produced by sires with the rat growth hormone transgene to ascertain the effect of the transgene in the progeny, 2) develop lines of mice with or without the transgene to initiate selection for increased adult weight and 3) assess frequency of mice with the transgene under of conventional breeding and selection.

DEVELOPMENT OF FOUR LINES OF MICE AND DNA ANALYSES

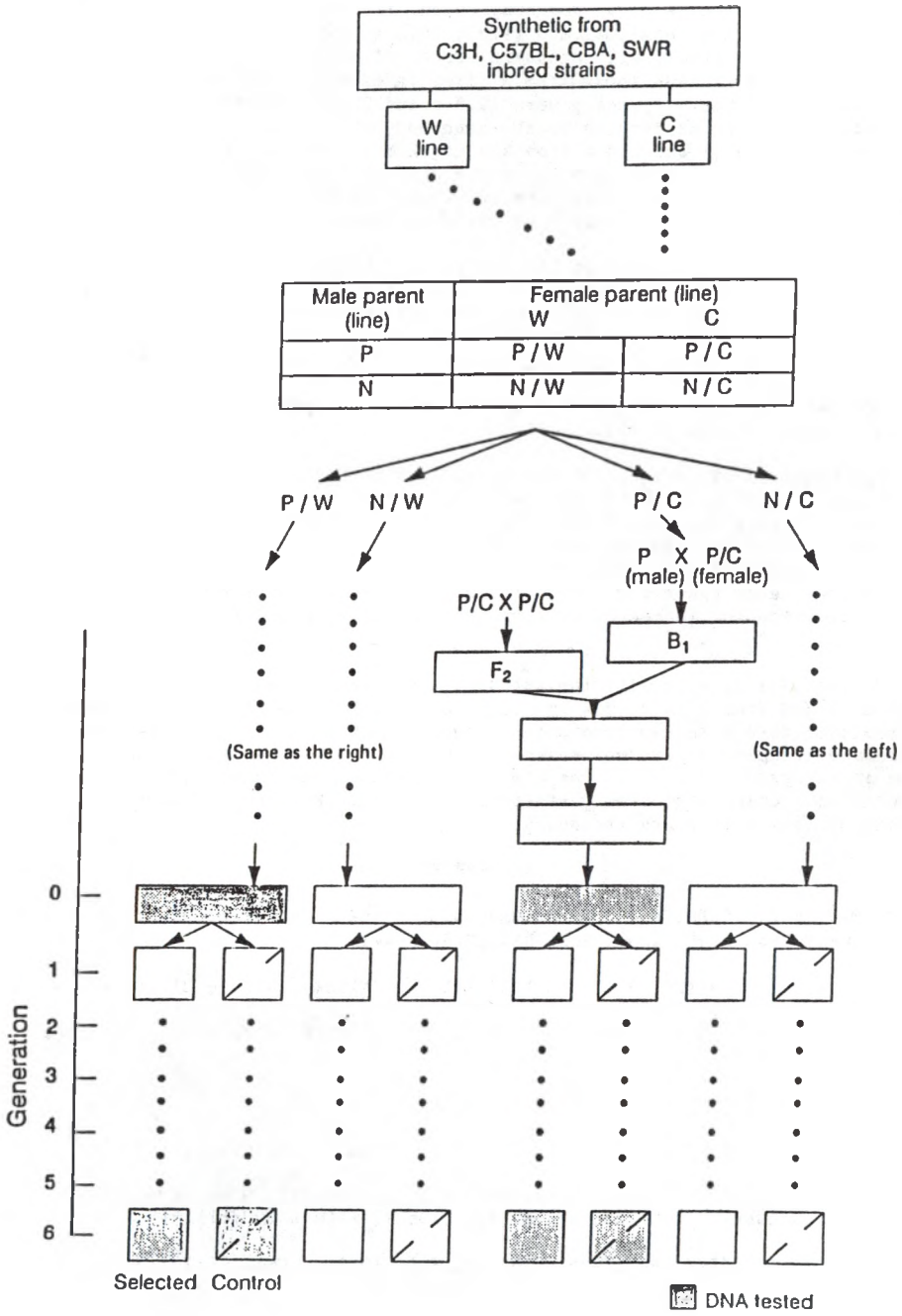
Mice: Five males and five females (P) with the MT-rGH transgene (Palmiter et al. 1982a) and five males and five females (N) without the transgene were obtained from the University of Pennsylvania (generous gift of Dr. R.L. Brinster). The P mice, now officially designated as Tg (Mt-1,GH)BRi2, were at the F_2 generation from the mating between C57BL/6 and SJL inbred strains while the N mice were at the F_1 or F_2 generation from mating the same strains. The P mice originated from "MGH-10 male" (Palmiter et al. 1982a) were hemizygous for the gene, each containing eight copies of the gene integrated at a single chromosome location. At the Animal Research Centre, they were pair mated within P or N at seven weeks of age. Thereafter, the original males with or without the transgene were mated with females of three lines (M, W and C) that were developed earlier at the Animal Research Centre.

The three lines were previously selected for increased 42-day body weight(line W), selected for increased nursing ability (line M), or unselected (control line, C). The lines originated from a population synthesized from four inbred strains (Nagai and Kristjansson 1970). After the line synthesis, selection was conducted for 12 generations. As a result, the three lines differed substantially in body weight at 42 days (Nagai et al. 1978). After selection, each line was maintained by random mating of 25 pairs every generation until Generation 82 when females of the three lines (W, M and C) were mated with P or N males at nine weeks of age. Resulting two-way cross (F_1) female progeny of lines W and C were mated with P or N males at seven weeks of age to produce backcross progeny (B_1). Two-way cross progeny (F_1), produced by second matings of PxW and PxC, were mated to produce their F_2 progeny. Using B_1 and F_2 progeny equally, four lines of P/W, N/W, P/C and N/C origin were developed (Fig. 1). They were maintained by random matings of 40 pairs per line for two generations. At the subsequent generation (generation 0), mice at 9 weeks of age were mated randomly (with the avoidance of full-sib matings) at the ratio of one male to two females. Resulting progeny were used to form selected and control sub-lines in each of the four lines. Each sub-line contained 40 pairs of breeders that were mated at random. Males and females in the selected sub-lines were selected for increased 42-day weight while those in the control sub-lines were randomly selected at 42 days. Each sub-line was maintained by random mating of 60 pairs after generation 2.

Throughout this experiment, males cohabited with females for 14 days and then removed. At parturition, litter size was standardized to eight after generation 0. Body weight of two-way cross (F_1) progeny were recorded individually at adulthood (42 days). The mice were maintained in a facility with controlled temperature and relative humidity. Pelleted feed and water, with no added metal ingredients (e.g. zinc), were supplied *ad libitum*.

Analysis of DNA: Presence or absence of the rat growth hormone transgene was examined using tails from 40 male breeders at generations 0 and 6. Slot blot hybridization procedures were applied as described by Palmiter et al. (1982b) and Brinster et al. (1985), modified by using a 350 bp XhoI-puvI fragment of rat growth hormone gene as probe.

Fig. 1 Breeding of four lines of mice



RESULTS AND DISCUSSION

Least squares means of 42-day body weight (sexes combined) of two-way cross progeny were 29.2 g (PxM), 31.6 g (PxW), 26.5 g (PxC), 25.1 g (MxM), 27.2 g (MxW) and 22.8 g (MxC), with standard errors of about 0.5 g. Progeny from P sires were heavier than those from M sires in each of the three lines of dams. Mean 42-day weights of progeny were 19.9 g and 22.8 g for MxM and PxP, respectively. The difference in the mean body weight between the progeny from M x M (19.9 g) and those from M x C (22.8 g) reflects the difference in the genetic background between the M and C lines (two inbred strains vs. four inbred strains for synthesizing the original genetic stocks), the difference in the age of dams (two weeks) plus possible heterotic effects of M x C.

Effects of age of sires on body weight of their progeny were assumed to be negligible (Nagai and Hickman, 1974). Sires carrying the transgene increased the mean 42-day body weight of progeny by 20% in progeny of dams of the same original inbred strains (23.9 g for PxP vs. 19.9 g MxM) and by 16% consistently in the population synthesized from four inbred strains (29.2 g vs. 25.1 g in M, 31.6 g vs. 27.2 g in W and 26.5 g vs. 22.8 g in C). In the latter case, heterosis due to the mating of the two populations of different origin may have been involved.

The analysis of variance of 42-day body weight for the two-way cross progenies revealed that the differences in actual 42-day weight were significant ($P<0.05$) between P and M sires, among the three lines (M, W and C) of dams and between the two sexes. The difference between the two male types (P and M) reflects the effect of the rat growth hormone transgene while the difference among the three lines of dams (M, W and C) indicates the effect of past selection for increased milk production (in M) or adult body weight (in W).

Frequencies of mice with the rat growth hormone transgene at generations 0 and 6 ranged from 2.5% to 30% in P-derived selected and control sub-lines, and were lower than expected frequencies (approx. 50%). Reasons for the low observed frequencies are not known. Mice with the transgene could have been low in fitness and/or the transgene could have been disintegrated in the course of transmission from parents to offspring. Further experiments that focus on this matter are necessary.

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