CYTOPLASMIC EFFECTS ON SELECTION RESPONSE FOR INCREASED GROWTH RATE IN MICE

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ABSTRACT

To determine if cytoplasmic effects have contributed to long-term selection response for increased growth rate in mice, reciprocal cross matings were made between an unselected control line (ICR) and a line (M16) derived from ICR by long-term selection for high post-weaning weight gain from 3 to 6 wk of age. Embryos were recovered 2 to 4 d following mating and transferred to pseudopregnant F1 (DBA/2NCrlBR × C57BL/6NCrlBR) females. Thus, all embryos developed in similar uterine and postnatal maternal environments. A total of 122 M16 × ICR and 123 ICR × M16 mice was produced, representing 19 litters from each cross. Litters were standardized at birth to five to seven pups. Litter weights at birth and 1 wk were recorded. Body weights at 2, 3, 4, 5 and 6 wk and weight gain from 3 to 6 wk were obtained. Weights of liver, kidneys, and sc and epididymal fat pads of males were obtained at 6 wk. Females were mated at 8 wk, and litter size at birth was recorded. Least-squares procedures were used to test for differences between reciprocal crosses for all traits. Body weight at 4 wk was higher (P<.05) for mice with ICR cytoplasm. No other significant differences were detected. There was no evidence that cytoplasmic effects influenced direct or correlated responses to long-term selection for increased postweaning weight gain. (Key Words: Cytoplasmic Inheritance, Selection Responses, Growth, Mice.)

Introduction

Response to selection for quantitative traits is assumed to be due primarily to nuclear genes (Falconer, 1981). Cytoplasmically inherited traits are, for the most part, ignored or assumed to be inconsequential (Rothschild and Ollivier, 1987). Recent evidence suggests that cytoplasm may have a significant effect on quantitative traits such as milk production, growth and reproduction in livestock (Dzapo et al., 1983; Bell et al., 1985; Huizinga et al., 1986; Toelle et al., 1986; Tess et al., 1987). Brumby (1960) found that the cytoplasm of a strain of mice selected for small body size enhanced growth compared with the cytoplasm of a large strain. The objective of the present study was to determine if cytoplasm and its constituents such as mitochondria have contributed to selection response for postweaning gain in mice.

Materials and Methods

Experimental Design. The extent to which cytoplasm may have contributed to selection response for high postweaning gain in mice was tested by performing reciprocal crosses between the selected and control lines. Embryos from the reciprocal crosses were transferred to uniform recipients (F1 females from the cross of two inbred strains) to provide for a similar uterine and postnatal environment (figure 1). Differences between reciprocal crosses would be indicative of cytoplasmic effects on the growth and reproductive traits measured. The sex × reciprocal cross interaction mean square was used to test for sex-linkage.

Strains of Mice. Embryos were obtained from reciprocal crosses of a random-bred line (ICR) of albino mice and a line (M16) derived from ICR by long-term selection for high postweaning gain from 3 to 6 wk of age (Eisen, 1975). These lines were chosen because M16 greatly exceeds ICR in body weight, adiposity, organ weights and litter size (Eisen et al., 1973, 1977; Eisen and Leatherwood, 1978; Eisen, 1986). The reciprocal crossbred progeny were designated as M16 × ICR and ICR × M16, where the sire line is first.
Figure 1. Design of experiment to estimate cytoplasmic effects on selection response in mice. ICR: random-bred line; M16: long-term selection line; BDF$_1$: DBA/2CrI BR × C57BL/6NCrI BR.

Uniform recipient female mice were BDF$_1$ black mice (DBA/2CrI BR × C57BL/6NCrI BR)$^3$. These females were mated to albino vasectomized studs to induce pseudopregnancy. Transferred embryos from reciprocal crosses would result in albino pups; progeny from inappropriately vasectomized studs would be pigmented. No pigmented progeny were obtained.

**Experimental Procedure.** Embryo donors and recipients were maintained on Purina Mouse Chow$^4$. Embryo donors were not superovulated. Reciprocal crossbred embryos from each donor female were surgically transferred to an individual pseudopregnant recipient (figure 1). Embryos were at the one-cell, two-cell or blastocyst stage, depending on availability of recipients. One-cell and two-cell embryos were surgically transferred to oviducts and blastocysts to uteri. Recipients were either mated on the same day as donors (one-cell) or 1 d later (two-cell and blastocyst).

At birth, litter size was standardized to five to seven pups, and the litter was weighed. Litter weights were recorded at 1 wk of age. At 2 wk of age, individual weights were recorded. Individual weights were recorded at 3 wk; pups were weaned, separated by sex and maintained on Purina Lab Chow$^4$. Individual weights were recorded at 4, 5 and 6 wk of age. At 6 wk, the males were euthanized by cervical dislocation, and weights of sc hindlimb and epididymal fat pads, kidneys and liver were recorded. Females were backcrossed to their sire line at 8 wk of age; they were maintained on Purina Mouse Chow, and litter size was recorded at birth.

### Statistical Analysis

All data were analyzed by least-squares procedures for unequal subclass numbers (Harvey, 1979). The statistical model for weekly individual body weights from 2 to 6 wk of age included an overall mean, a fixed reciprocal cross effect, a random litter within reciprocal cross effect, a fixed sex effect, a sex × reciprocal cross interaction effect, covariates and a residual effect. The covariates examined included age of donor female, stage of embryo at transfer and litter size at birth and weaning.

The litter mean square was used as an error term for testing reciprocal crosses and covariates. The other mean squares were tested by the residual mean square.

For traits measured either on a litter basis (birth weight, 1 wk body weight) or in one sex (fat pad weights, organ weights, litter size), sex and sex × reciprocal effects were omitted. The fat pad and organ weights were analyzed either as percentages of body weight or by adjusting fat pad or organ weight for the covariate body weight. In the latter analyses, all weights were transformed to natural logarithms to provide a linear relationship between dependent and independent variables.

### Results

The overall success of embryo transfer was 55% based on number born as a percentage of number of embryos transferred, which is an acceptable survival rate. Postnatal survival of reciprocal cross progeny was high (99%), with no evidence of differential mortality between reciprocals.

Least-squares means of weekly body weights are listed in table 1. Weekly body weights of males from 3 to 6 wk of age were larger (P<.01) than those of females. Body weight means of reciprocal crosses were significantly different at 4 wk only; however, this difference was relatively small and had disappeared by 6 wk of age. The reciprocal effect for 3 to 6 wk postweaning gain, the selection criterion in the M16 line, was not significant. Sex × reciprocal cross interactions were not significant for weekly body weights or postweaning gain.

Fat pad and organ weights in male mice as a percentage of body weight or adjusted for body weight were not significantly different between reciprocal crosses (table 2).

Least-squares means of first parity litter size at birth in reciprocal F$_1$ females did not differ (P>.05); the respective means were 15.11 ± .48

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$^3$ Charles River Breeding Laboratories, Wilmington, MA.

$^4$ Ralston Purina Co., St. Louis, MO.
TABLE 1. LEAST-SQUARES MEANS OF WEEKLY BODY WEIGHTS FROM BIRTH TO 6 WK OF AGE AND POSTWEANING GAIN

<table>
<thead>
<tr>
<th>Weight, g</th>
<th>Reciprocal F₁, a</th>
<th>M16 × ICR</th>
<th>ICR × M16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Birth wt</td>
<td>1.73</td>
<td>.031</td>
<td>1.82</td>
</tr>
<tr>
<td>1 wk</td>
<td>6.21</td>
<td>.122</td>
<td>6.11</td>
</tr>
<tr>
<td>2 wk</td>
<td>10.95</td>
<td>.149</td>
<td>10.83</td>
</tr>
<tr>
<td>3 wk</td>
<td>17.72</td>
<td>.214</td>
<td>17.15</td>
</tr>
<tr>
<td>4 wk</td>
<td>29.09</td>
<td>.264</td>
<td>28.28</td>
</tr>
<tr>
<td>5 wk</td>
<td>35.61</td>
<td>.308</td>
<td>34.97</td>
</tr>
<tr>
<td>6 wk</td>
<td>38.33</td>
<td>.301</td>
<td>38.27</td>
</tr>
<tr>
<td>3–6 wk gain</td>
<td>20.65</td>
<td>.260</td>
<td>21.08</td>
</tr>
</tbody>
</table>

aSire X dam; number of mice per cross varied from 120 to 123; 19 litters per cross.
bPooled across sexes; body wt at birth and 1 wk were calculated from litter means; 1-wk wt adjusted by covariance analysis for litter size and no. at measurement; 2-wk wt adjusted for litter size and no. suckling pups; 3-wk wt adjusted for litter size, no. suckling pups and age of donor female; 4-wk and 5-wk adjusted for litter size and age of donor female; 6-wk wt adjusted for litter size.

Reciprocal crosses differ (P<.05).

(n = 51) and 14.89 ± .48 (n = 62) for M16 × ICR and ICR × M16.

Discussion.

Direct and correlated responses in selected lines of mice such as M16 may be the result of additive effects of nuclear genes either through direct effects, uterine maternal or postnatal maternal effects as well as cytoplasmic maternal genes. A previous study has shown that nuclear direct and maternal genetic effects were present for 3-wk and 4-wk body weight and 3 to 6 wk feed consumption and feed efficiency (Deodato et al., 1982). However, in that study, cytoplasmic effects could not be separated from uterine effects.

TABLE 2. LEAST-SQUARES MEANS OF MALE 6-WK FAT PAD AND ORGAN WEIGHTS AS A PERCENTAGE OF BODY WEIGHT AND AS ABSOLUTE WEIGHTS

<table>
<thead>
<tr>
<th>Trait b</th>
<th>Reciprocal F₁, a</th>
<th>M16 × ICR</th>
<th>ICR × M16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Epididymal fat pads</td>
<td>2.32c</td>
<td>.08</td>
<td>2.41</td>
</tr>
<tr>
<td>Subcutaneous fat pads</td>
<td>1.12</td>
<td>.04</td>
<td>1.20</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.62</td>
<td>.02</td>
<td>1.60</td>
</tr>
<tr>
<td>Liver</td>
<td>6.99</td>
<td>.16</td>
<td>6.70</td>
</tr>
</tbody>
</table>

aSire X dam; numbers of mice were 66 for M16 × ICR and 56 for ICR × M16; 19 litters per cross.
bEpididymal fat pad wt adjusted by covariance analysis for age of donor female, stage of transfer and litter size; sc fat pad wt adjusted for age of donor female and stage of transfer; kidney wt adjusted for stage of transfer.
cUpper values are weights as percentages of body weights. Lower values are weights transformed back to original gram units after analysis as natural logarithm 6-wk fat pad or are organ weights adjusted by covariance analysis for natural logarithm 6-wk body weights.
and postnatal maternal effects. The experimental design in the present report allows evaluation of maternal cytoplasmic genetic effects while holding uterine and postnatal maternal environments constant. However, nucleus × cytoplasm interaction would not be detectable.

Cytoplasmic variation in quantitative traits within a breed has been reported in dairy cattle (Bell et al., 1985; Huizinga et al., 1986), beef cattle (Tess et al., 1987) and swine (Toelle et al., 1986). These studies were based on variation among founder females within a population. In dairy cattle, 102 cytoplasmic lines were examined by tracing the pedigrees of 4,461 cows (Bell et al., 1985). Analysis of these data led to estimates that 1.8% to 3.5% of the total variation in several milk production traits were due to cytoplasmic effects. However, a recent study found no evidence for cytoplasmic inheritance of milk production traits in dairy cattle (Reed and Van Vleck, 1987). Kennedy (1986) cautioned that significant cytoplasmic effects may reflect additive genetic effects unaccounted for by statistical models used.

Reciprocal cross effects (suggestive of cytoplasmic effects) have been demonstrated in cattle and swine. Klindt and Maurer (1986) transferred reciprocal cross embryos of Angus and Red Poll cattle into two types of recipient dams. Of the traits examined, only basal prolactin concentration was reported to have shown significant reciprocal differences. However, the whole-plot error mean square used to test for reciprocal differences was about one-fourth as large as the within-plot error mean square. A pooled error mean square would not have declared the reciprocal effect significant. Reciprocal cross differences between German Landrace and German Edelschwein pigs have been reported (Dzapo et al., 1983). In these crosses, significant effects were noted for lean:fat ratio (Dzapo et al., 1983), mitochondrial oxidative phosphorylation (Dzapo and Wassmuth, 1983) and activity of mitochondrial respiratory chain enzymes (Dzapo and Wassmuth, 1984).

The present report, using reciprocal crosses and embryo transfer to genetically uniform recipient females, indicates that cytoplasmic effects probably have not contributed to the direct and correlated long-term selection responses seen in the M16 line. Of the traits measured, only body weight at 4 wk was significantly different between reciprocal crosses. Differences that may have existed in the mitochondrial genome of the ICR base population have not contributed to the selection response. Considering that most laboratory mice that have been examined share a common mitochondrial DNA profile based on restriction fragment length analysis (Yonekawa et al., 1982; Ferris et al., 1983), mitochondrial differences between the M16 and ICR lines seem unlikely. However, these lines have not been subjected to such analysis.

Sixteen full-sib families were sampled from the original ICR stock, and selection in M16 was carried out within full-sib families. Under this selection scheme, expected direct or correlated responses in nuclear-mediated maternal effects arise because of a covariance between additive direct and additive maternal nuclear effects (Eisen et al., 1973). If selection were strictly within full-sib families, there would be no opportunity for selection among cytoplasmic DNA (Rothschild and Ollivier, 1987). However, families were lost during selection because the proportion of infertile matings reached about 40% by generation 13 (Eisen et al., 1973), and this resulted in selection among families that continued for an additional 27 generations.

Contrary to the present report, Brumby (1960) found a significant cytoplasmic effect in reciprocal crosses between large and small strains of mice. The cytoplasm of the small strain animals enhanced body size relative to that of the large strain. This result is surprising, considering that selection also was practiced within full-sib families and considering cited evidence for absence of mitochondrial restriction fragment length polymorphism. In addition to cytoplasmic factors, Brumby (1960) suggested three further explanations for the reciprocal differences: the early maternal environment of the ova prior to transfer, differential mortality of ova actually implanted and a chance occurrence. Differences in experimental protocol may also account for the conflicting findings of the present study and that of Brumby (1960). The present study used adult females with no exogenous hormone priming, whereas Brumby used immature donor females and superovulation of both donor and recipient females. In addition, Brumby used unselected control females as embryo recipients and nurse dams. These females possess much genetic variation and would not provide the uniformity of prenatal and postnatal maternal environments achieved in the present
study with the F₁ cross of two inbred lines, especially considering the small sample size involved.

A source of new variation in the mitochondrial genome could be recurring mutation. Hill (1982) has shown that mutation in the nuclear genome theoretically can increase genetic variance and thus can contribute to greater genetic response in long-term selection studies. One might expect a similar contribution from mitochondrial genes. However, the lack of restriction fragment length polymorphisms among mitochondrial DNA of inbred lines of laboratory mice suggests that the mitochondrial DNA is highly conserved in this species (Yonekawa et al., 1982; Ferris et al., 1983), and enhanced response from mutation-derived mitochondrial genetic variation seems unlikely.

Sex-linkage can be an additional cause of reciprocal cross differences, but this reciprocal effect would be expected only in male progeny, where the differential contribution of the M16 and ICR X-chromosomes would be expressed. The absence of any sex by reciprocal cross interactions for weekly body weights from 2 to 6 wk and gain 3 to 6 wk postweaning indicates that sex-linkage was not likely to be involved. Brumby (1960) also reported an absence of sex-linkage in the determination of body size in mice.

Cytoplasmic inheritance and its effects should be considered in genetic selection experiments when significant variation for such traits (i.e., mitochondrial DNA) exists in the base population. This may be the case for cattle and swine. However, response to selection in laboratory strains of mice probably will not be influenced by cytoplasmic effects because significant cytoplasmic variation apparently does not exist between these strains. Our results with reciprocal crosses between ICR and M16 support this hypothesis.

**Literature Cited**


