C3H mice have higher average ploidy megakaryocytes than all other mouse strains tested, but the mode of inheritance of this anomaly is unknown. Therefore, to clarify the genetics of high ploidy megakaryocytes in C3H mice, we measured megakaryocyte DNA content from both male and female offspring from F1, as well as backcross matings. In all, offspring from seven different matings of mice were studied: (1) C57BL X C57BL (the first strain listed is the male parent in each case), (2) B6C3F1 (offspring from C57BL X C3H mating) X C57BL, (3) C57BL X B6C3F1, (4) C57BL X C3H, (5) C3H X B6C3F1, (6) B6C3F1 X C3H, and (7) C3H X C3H. The polyploid megakaryocyte DNA content distributions of the offspring from these matings show that C3H mice have higher percentages of high ploidy megakaryocytes than did all other mice. Also, male mice had significantly higher percentages of high ploidy (32N and 64N) megakaryocytes than did female mice for all matings, except backcross mating no. 6. The megakaryocyte DNA content for individual offspring of a given backcross appeared to form a single, continuous distribution, rather than segregate into two distinct groups, suggesting that the higher megakaryocyte DNA content of C3H mice is caused by involvement of multiple alleles. This conclusion is further supported by our finding that the frequency of high ploidy megakaryocytes among offspring of the various matings was related to the proportion of C3H genotype contributed by the parents, ie, average megakaryocyte DNA content increased linearly (r2 = .88 for male mice and .84 for female mice, P < .0001) with increasing C3H gene dosage; the correlations for both male and female mice were essentially parallel (slope = 0.08 and 0.09, respectively). In addition, we found an effect of genomic imprinting on megakaryocyte DNA content in backcross offspring. The genetic imprinting was characterized by the female parent having a greater influence on the offspring’s megakaryocyte DNA content than the male parent. Although the overall genetic makeup was the same, female offspring from backcross no. 6 (in which the female was C3H) had higher average megakaryocyte ploidy values than those from backcross no. 5 (in which the female was B6C3F1). The same phenomenon was observed when comparing the results from backcrosses no. 2 and 3, ie, male offspring from mating no. 3 (B6C3F1 female parent) had a higher average polyploid megakaryocyte DNA content than did male offspring from backcross no. 2 (C57BL female parent). Thus, we find that a number of factors influence megakaryocyte DNA content, including genomic imprinting and male sex hormones; however, we conclude that the higher megakaryocyte DNA content of C3H mice results from the interaction of multiple alleles that are additive. We hypothesize that a larger dosage of these genes in the C3H mouse is responsible for the higher DNA content of their megakaryocytes.

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ploidy in C3H mice compared with other mouse strains. However, if the DNA values of megakaryocytes of the backcross offspring fit a single, continuous distribution, the mode of inheritance of the higher C3H megakaryocyte ploidy is likely caused by multiple genes. We confirm herein that C3H mice had higher average ploidy megakaryocytes than C57BL mice, and that F1 offspring of C57BL × C3H crosses have mean ploidy values intermediate between those of parents. The average megakaryocyte ploidy values for offspring of a given backcross showed a single, continuous distribution. In addition, when offspring of all crosses were considered, average megakaryocyte polyploid DNA content increased linearly with increasing C3H genetic content. These results suggest that multiple alleles are responsible for the higher megakaryocyte DNA content of C3H mice. Male mice had higher ploidy megakaryocytes than did female mice. Another unexpected and potentially important finding is that the female parent had a greater influence than the male parent on megakaryocyte DNA content of backcross offspring, indicating a role for genomic imprinting in the inheritance of megakaryocyte ploidy.

**MATERIALS AND METHODS**

**Animals.** A total of 219 mice, including both male and female offspring, from the mouse matings shown in Table 1 were studied. Five- to 6-week-old breeder mice, ie, C57BL/6NHSD (C57BL), B6C3F1/HSD (B6C3F1; offspring from C57BL male and C3H female matings), and C3H/HENHSD (C3H), were purchased from Harlan Sprague-Dawley (Indianapolis, IN). The mice were mated in triplo culture, adenosine, theophylline contents, the marrow cells were collected by flushing a femur and tibia with 1.0 mL of CATCH medium (solution that contains citrate, adenosine, theophylline in calcium- and magnesium-free Hanks' medium with bovine serum albumin [BSA]) containing DNAse into plastic tubes. The cell suspensions were incubated

![Table 1. Genetic Background of Parents of Mice Used in Study](image)

<table>
<thead>
<tr>
<th>Mating</th>
<th>Male × Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C57BL/6NHSD × C57BL/6NHSD</td>
</tr>
<tr>
<td>2</td>
<td>B6C3F1/HSD × C57BL/6NHSD</td>
</tr>
<tr>
<td>3</td>
<td>C57BL/6NHSD × B6C3F1/HSD</td>
</tr>
<tr>
<td>4</td>
<td>C57BL/6NHSD × C3H/HENHSD</td>
</tr>
<tr>
<td>5</td>
<td>C3H/HENHSD × B6C3F1/HSD</td>
</tr>
<tr>
<td>6</td>
<td>B6C3F1/HSD × C3H/HENHSD</td>
</tr>
<tr>
<td>7</td>
<td>C3H/HENHSD × C3H/HENHSD</td>
</tr>
</tbody>
</table>

For measurement of megakaryocyte DNA content, the marrow cells were collected by flushing a femur and tibia with 1.0 mL of CATCH medium (solution that contains citrate, adenosine, theophylline in calcium- and magnesium-free Hanks' medium with bovine serum albumin [BSA]) containing DNAse into plastic tubes. The cell suspensions were incubated with a saturating concentration of rabbit antimouse platelet serum (RAMPS) for 30 minutes at 4°C, followed by three washings in 3 mL of CATCH medium (160g for 5 minutes at 4°C). Each sample was labeled with fluorescein-conjugated goat antirabbit Ig F(ab')2 fragments (Tago, Burlingame, CA). Before measurement on an EPICS 753-Flow Cytometer (Coulter Electronics, Hialeah, FL), the cells were resuspended in a hypotonic solution of propidium iodide as previously described.

1. From 301 × 10^3 to 801 × 10^3 (average, 542 × 10^3) propidium-iodide-positive cells were examined, and the DNA content of from 526 to 2,328 (average, 973) RAMPS-positive (FITC-positive) cells with DNA content ≥8N was analyzed for each mouse. The mean frequency of FITC-positive cells/1 × 10^5 propidium-iodide-positive bone marrow cells for each mating is shown in Table 2. The results show no sex-related differences. Significantly greater (< .01) frequencies of FITC-positive cells were found in male offspring of mating no. 2 than male mice from matings no. 3 and 4. Female mice from mating no. 1 had higher values than did female offspring from mating no. 6. However, no relationship of C3H genotype and the frequency of FITC-positive cells/nucleated bone marrow cell was found.

**Statistics.** Student's t-test was used to determine statistical differences between the means due to the sex of the mice. The megakaryocyte DNA distributions were analyzed by analysis of variance with a general linear mixed models (GLMM) procedure after the percent frequency of individual polyploid classes was calculated for each mouse. Within each ploidy class, statistical analysis was determined using Tukey's Studentized Range test. Average polyploid megakaryocyte DNA content was calculated by use of geometric means from individual mice. A linear correlation coefficient test was used to evaluate the relationship of both the proportion of individual ploidy classes and average polyploid megakaryocyte DNA content versus mouse genetic content.

**RESULTS**

Figure 1 compares average megakaryocyte DNA content distributions of both male and female offspring from the 7 different matings. Male mice had higher percentages of high ploidy (32N and 64N) megakaryocytes than did female mice (P < .05 to P < .0005), except in mating no. 6, in which the differences were not significantly different. Statistical evaluation (Table 3) of the data by analysis of variance and Tukey's Studentized Range test show that offspring with 100% C3H genotype (mating no. 7) had a lower frequency of 8N and 16N megakaryocytes (P < .01), but higher percentages of 32N and 64N megakaryocytes (P < .01) than mice with 0% to 25% C3H genotype (matings no. 1 through 3). This trend was followed by offspring from matings no. 5 and 6 (75% C3H genotype), in which significantly (P < .01) smaller proportions of 8N to 16N megakaryocytes were found. Also, significantly (P < .01) greater numbers of 32N to 64N megakaryocytes were present in mice with 75% C3H genotype than in C57BL mice (0C3H genotype, mating no. 1), except when comparing the percentages of 64N megakaryocytes in female offspring from mating no. 1 with those from mating no. 5. In this case, the frequency of 64N megakaryocytes was significantly different, but at a lower level of significance (P < .05). Conversely, C57BL mice (mating no. 1) had higher percentages (P < .01) of low ploidy megakaryocytes (8N and 16N) and lower numbers of high ploidy (32N to 64N) megakaryocytes than mice from matings no. 5 through 7 (75% to 100% C3H genotype). All ploidy classes for each sex showed a significant correlation coefficient (P < .0001) when comparing frequencies of megakaryocyte ploidy class to the percentage of C3H genotype in the offspring (Table 3), except when comparing the data on 128N megakaryocytes. The reason for the lack of correlation in this case is probably because the numbers of megakaryocytes were too low for analysis.

Average polyploid megakaryocyte DNA contents derived from geometric means of individual mice of the 7 different
matings (Table 1) are shown in Fig 2. Male mice had higher 
(P < .05) average polyploid megakaryocyte DNA content than did female mice in all cases except back-
cross mating no. 6 (B6C3F1 male x C3H female), in which the differences were not significant. Statistical analyses of the other comparisons of data are presented in Table 2. The data show that both male and female mice from mating no. 7 (C3H mice) had significantly higher (P < .01) megakaryo-
cyte DNA content than mice from all other matings (no. 1 through 6). In addition, when comparing mating no. 1 (C57BL male) with all other mice, there was significantly (P < .01) lower average megakaryocyte DNA content, except mice from mating no. 2 (B6C3F1 x C57BL). The average polyploid megakaryocyte DNA content of male offspring from mating no. 4 (C57BL x C3H) was significantly higher (P < .01) than values from male mice with 25% C3H geno-
type (matings no. 2 and 3), but significantly lower (P < .01) than mice with 75% C3H genotype (matings no. 5 and 6). In female mice, the division was not so clear. Female mice from mating no. 4 (C57BL x C3H) had higher (P < .01) average megakaryocyte DNA content than female mice from matings no. 2 and 3 (25% C3H genotype) and lower values (P < .01) than female offspring from mating no. 6 (B6C3F1 x C3H). However, average ploidy values of female mice from mating no. 4 (C57BL x C3H) were not different from values of female mice from mating no. 5 (C3H x B6C3F1).

Figure 3 shows the distribution of geometric means of megakaryocyte DNA content of individual male and female mice from backcross matings no. 2 and 3 and 5 and 6
(B6C3F1 x C3H or C57BL and C3H or C57BL x B6C3F1). As shown, the values do not segregate into two groups, but appear to be from a single continuous distribution.

An unexpected finding was that male mice from mating no. 3 (C57BL males x B6C3F1 females) had higher (P < .01) average polyploid megakaryocyte DNA content than did male mice from mating no. 2 (B6C3F1 males x C57BL females), ie, mice with the same genetic background (Fig 2 and Table 2). Although male mice did not differ when comparing the average DNA content of megakaryocytes from offspring of matings no. 5 versus 6, female mice from mating no. 6 (B6C3F1 males x C3H females) had higher (P < .01) average polyploid megakaryocyte DNA content than did female mice from mating no. 5. In both cases, the female parent had a greater influence on average polyploid mega-
karyocyte DNA content than did the male parent.

Also, when the data on megakaryocyte DNA content from Fig 2 are expressed as a percentage of C3H genotype (Table 2), there was a highly significant (P < .0001) correlation coefficient between the average polyploid megakaryocyte DNA content and percentage of C3H genotype for both male and female mice. The slopes of the lines (0.08 and 0.09, respectively) were significantly (P < .0001) altered, and the r2 values were .88 and .84 for male and female mice, respectively. These data serve to reinforce the conclusion that both male and female C3H mice have higher average polyploid megakaryocyte DNA content than do C57BL mice, and that the proportion of high ploidy megakaryocytes is related to C3H gene dosage.

**DISCUSSION**

The present report demonstrates that the mode of inheri-
tance of high ploidy megakaryocytes in C3H mice is linear with increasing amounts of C3H genotype in parents. More-
ever, the higher DNA content of C3H megakaryocytes is probably caused by multiple genes. Male mice had higher ploidy megakaryocytes than female mice did in all cases, except when comparing male and female offspring from male B6C3F1 and female C3H matings. In addition, we show that female parents have a greater influence on the DNA content of offspring megakaryocytes than do male parents. This finding appears to be an example of genomic imprinting, as described by Moore and Haig.

In agreement with the present report, we showed pre-
found in the percentages of the various polyploid DNA classes. In particular, CBA, SWR, and A/J strains had megakaryocyte DNA distributions similar to those of C57BL × C3H hybrids. In the present report, we also found intermediate modal ploidy values for megakaryocytes from B6C3F1 mice (22.2N average polyploid megakaryocyte DNA content) as compared with that of C57BL (19.2) and C3H (26.9) mice. In addition, the data show that offspring from backcross matings (ie, B6C3F1 × C3H or C57BL, and C3H or C57BL × B6C3F1) had intermediate megakaryocyte DNA values and that the data fit a single, continuous distribution. These findings suggest that multiple genes that have an additive effect control megakaryocyte DNA content in mice, and that the C3H mouse has a higher dosage of these genes than other mouse strains.

Previously, we showed sex- and strain-related differences in the megakaryocyte DNA content of mice. In that work, we used castrated male and oophorectomized female C3H and BALB/C mice, along with suitable intact controls, and found that intact male C3H mice had higher percentages of high ploidy megakaryocytes than did their neutered counterparts. In addition, castrated BALB/C mice had higher percentages of low ploidy megakaryocytes than did intact BALB/C male mice. Likewise, intact BALB/C male mice had higher proportions of high ploidy megakaryocytes than did their neutered counterparts. Because we found a shift to a lower ploidy class after castration of male mice, we concluded that male sex hormones cause an increase in DNA content of megakaryocytes. In agreement with these findings, the present work showed a significantly higher average polyploid megakaryocyte DNA content in male mice compared with values from female mice in offspring from all matings, except mating no. 6. These findings support the conclusion that male sex hormones elevate DNA content of megakaryocytes. Why male mice from mating no. 6 did not have higher megakaryocyte DNA values than female mice is unknown, but the fact that offspring from this mating had 75% C3H genotype and the maternal genotype was 100% C3H may indicate that maternal gene dosage overshadows sex hormone influence on megakaryocyte ploidy. However, if this were the case, the male mice from mating no. 7 should not have had higher megakaryocyte DNA content. Hence, another hypothesis is required to explain this finding.

There is a possibility that the cause of the higher ploidy in the C3H mouse is a result of a cytoplasmic maturational defect; thus, the higher DNA content of megakaryocytes in C3H mice might be caused by a secondary, compensatory response to defective megakaryocyte cytoplasmic development. In support of this hypothesis is the finding that platelet counts are actually lower in mice with higher ploidy megakaryocytes. However, our previous study showed that mature C3H megakaryocytes had normal ultrastructure, and recent unpublished data on the same mice used in the present study show that megakaryocytes become larger as DNA content increases.

The term genomic imprinting refers to cases in which genes have differential expression depending on the sex of the parent from which they are inherited, ie, specific genes are under transcriptional regulation via parental imprinting.
### Table 3. Statistical Analyses of Means for Each Ploidy Class of Data Presented in Fig 1

<table>
<thead>
<tr>
<th>Ploidy Class</th>
<th>Matings</th>
<th>% C3H Genotype of Offspring</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>8N</td>
<td>1</td>
<td>0</td>
<td>7.6a</td>
<td>12.2a</td>
<td>61.1a</td>
<td>66.6a</td>
<td>29.5a</td>
<td>19.7a</td>
<td>1.5a</td>
<td>1.2a</td>
<td>0.3a</td>
<td>0.3b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25</td>
<td>8.8a</td>
<td>10.4a</td>
<td>58.8a,b</td>
<td>64.8a</td>
<td>30.1a</td>
<td>23.3a,b</td>
<td>2.0a</td>
<td>1.1c</td>
<td>0.3a</td>
<td>0.3b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>25</td>
<td>6.0a,b</td>
<td>9.5a</td>
<td>52.0a</td>
<td>59.8a</td>
<td>38.1a</td>
<td>28.5a</td>
<td>2.8a</td>
<td>1.7e</td>
<td>0.4a</td>
<td>0.5a,b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>50</td>
<td>4.2a,b</td>
<td>6.0b</td>
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<td>49.3b</td>
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<td>75</td>
<td>4.0a,b</td>
<td>6.0b</td>
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<td>46.0a</td>
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<td>3.6a,b</td>
<td>0.3a</td>
<td>0.6a,b</td>
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</tr>
<tr>
<td></td>
<td>6</td>
<td>75</td>
<td>4.0a,b</td>
<td>4.9b</td>
<td>35.5a</td>
<td>37.5c</td>
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<td>7</td>
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<td></td>
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</table>

For description of matings, see Table 1. Within each ploidy class, means with different superscripts were significantly different (P < .01) using Tukey's Studentized Range test. Correlation coefficient values were determined on individual mice within each ploidy class versus the proportion of C3H genotype (r²); the probability (P) of the slope of the regression line being different from 0 is shown in each column.

that results in the gene from one parent being expressed and the same gene from the other parent being repressed. Although we did not compare the DNA content of megakaryocytes from offspring of a mating of C3H male × C57BL female with B6C3FI mice (C57BL male × C3H female), we did compare both C3H and C57BL backcrosses on B6C3FI mice, and show significantly (P < .01) greater DNA content in megakaryocytes of offspring of both sexes if the female parent (B6C3FI) had a higher C3H genotype compared with matings in which the female did not have C3H genotype (C57BL). In other words, we show that the genes controlling megakaryocyte DNA content change their pattern of expression depending on whether they are maternally or paternally inherited.

The egg contributes virtually all of the zygotes' cytoplasm.15 Transcription factors are introduced with the sperm, with genetic modifications both before and after fertilization. Thus, factors that increase DNA content of megakaryocytes may arise in the cell's cytoplasm, or, alternatively, the paternal genes may introduce inhibitory factors that result in higher DNA content of the cell. This latter explanation is probably incorrect, because male mice have higher DNA content in their megakaryocytes than females. Based on the data presented herein, it seems possible that the presence of X chromosomes might increase the expression of genes that control DNA content of megakaryocytes, or it could be possible that the presence of a Y chromosome might act to repress the same genes. Regardless, it seems certain that the regulation of polyploid megakaryocyte DNA content in mice is via multiple genes that act in an additive manner, and that the C3H mouse has a higher dosage of these genes than other mouse strains.

In disagreement with the conclusions of the present report that multiple genes are responsible for controlling megakaryocyte DNA content computed from DNA distributions presented in Fig 1 from the various matings of C57BL, B6C3FI, and C3H mice. The first strain listed is the male parent in each case. Bars are the average of each treatment group or 1 SE and the numbers on the bars represent the number of mice per treatment. Values for (□) male mice were significantly higher than values for (○) female mice: *P < .05, **P < .005, ***P < .0005.
Megakaryocyte ploidy, a recent preliminary study,\textsuperscript{16} using a human Dami megakaryocytic cell line,\textsuperscript{17} reported that transfection of a single gene induced polyploidization of a diploid cell line (Chinese hamster ovary cell line). A single gene that was selectively activated in megakaryocytes was, therefore, postulated. Our data in mice point to the involvement of multiple genes in controlling megakaryocyte DNA content. Therefore, resolving this discrepancy and establishing the mode of inheritance of megakaryocyte ploidy will require additional work.

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Mode of inheritance of the higher degree of megakaryocyte polyploidization in C3H mice. I. Evidence for a role of genomic imprinting in megakaryocyte polyploidy determination

TP McDonald and CW Jackson