EFFECT OF HETEROSIS ON PERFORMANCE OF MICE ACROSS THREE ENVIRONMENTS1,2

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ABSTRACT

Performance of six genetic groups of mice was studied in three different environments in order to determine the effect of heterosis on consistency of performance in varied environments. Designed as a 3 x 6 factorial, data were analyzed using least squares analyses of variance. Genetic group effects, environmental effects, genetic group x environment interaction, and heterosis were examined for 42-d weight, age and weight at vaginal opening, ovulation rate (determined by total corpora lutea), number of implantations, and number of fetuses at 10 d of gestation. Weight at vaginal opening, ovulation rate, implantation rate and total number of fetuses exhibited significant heterosis. Regression of these traits against an environmental index (mean performance of all genetic groups over environments) provided an estimate of stability of performance. In general, genetic groups exhibiting heterosis expressed more consistent performance across environments than pure lines did. We concluded that a stability model could be used to aid in identification of lines with consistent performance for production traits in variable environments.

Key Words: Mice, Heterosis, Interactions


Introduction

Breeders have recognized that phenotype is a function of both genotype and environment and have approached genotype x environment (G x E) interaction in many ways (Pani and Lasley, 1972). The suggestion by Falconer (1952) that a single character under two different environments is not the same trait revolutionized G x E interaction studies. If the genetic correlation between two measures of the trait taken in different environments does not equal 1, then the trait is affected by G x E interaction. Regressions of means of a trait for different genetic groups across environments provide a measure of G x E interaction. Data regarding phenotypic stability, heterosis (hybrid vigor) and response to environmental change suggest that genetic groups expressing heterosis are less sensitive to environmental change than inbred genetic groups are (Mather, 1953). For example, parental genetic groups had greater variance than F1 genetic groups in Primula (Mather, 1950), Drosophila (Mather, 1953), maize (Sprague and Federer, 1951; Mather, 1949, 1953) and barley (Finlay and Wilkinson, 1963). This supports Lerner's (1954) conclusion that in outbreeding species heterozygotes are able to buffer environmental changes better than homozygotes.

The objective of this study was to verify the hypothesis that heterozygosity in mice is associated with an improved ability to maintain homeostasis in the face of environmental variation. Homeostasis was measured using stability parameters described by Eberhart and Russell (1966).

Materials and Methods

Three stocks of mice, Mus musculus (Hsd: (ICR)BR; Hsd: (ND4)(S)BR; Hsd:
Mice were mated to have litters born on
removed. Litters were weighed, ear-notched
three in suspended, stainless steel, wire-bottom
light:
all females to
approximately the same
were established by mating ICR and
room was equipped with overhead fluorescent
had ad libitum access to fresh food
bedding was changed twice weekly and
in a petri dish. Using two watchmaker forceps,
implantation sites and number of fetal pups
was made to
No
Statistical Analyses. Designed as a 3 × 6
factorial, data were analyzed using least
squares analyses of variance (Harvey, 1987).

4Harlan Laboratories, Indianapolis, IN.
5Purina Mills Formula Lab Chow #5008, St. Louis, MO.
6#16708, Scherer, Inc., Marshall, MN.
Model type I was used for each analysis. Effects of genetic group, temperature and the temperature × genetic group interaction were examined for all traits. In addition, covariates of age at weighing and weight at chamber entry were examined for 42-d weight. Significance levels of genetic group effects are biased because the residual mean square rather than sire within genetic group was used to test differences among genetic groups.

A pooled, within-class partial correlation between age at first plugging and age at vaginal opening (AVO) was calculated using SAS (1985). The model used included genetic group, temperature and their interaction. Heterosis for all traits was determined by comparing performance of pure line vs backcrossed genetic groups. Because different crosses were expected to yield different amounts of heterosis, a single contrast statement was used to appropriately weight the stability parameters that can be used to describe the performance of a genetic group over a series of environments.

Genetic groups intermediate. Temperature did not affect 42-d weight. Regression of 42-d weight on weight at chamber entry was significant (P < .001), as was regression on age at weighing (P < .01).

Only genetic group affected AVO and weight at vaginal opening (WV0). CF1 and CF1 maternal genetic groups were consistently lighter in WV0 (P < .05); CF1s averaged 13.2 g, vs 16.1 g for ICRs. CF1 mice reached AVO later (P < .01) than ICRs (27.8 vs 27.0 d); all crosses were intermediate.

Temperature × genetic group interaction affected AP (P < .01) but not weight at puberty (WP). ICRs reached puberty earlier than CF1s at both 8 and 15°C (48.3 vs 65.7 d, P < .01; 42.0 vs 47.3 d, P < .05); however, CF1s reached puberty earlier than ICRs at 22°C (40.5 vs 48.5 d, P < .05). CF1 maternal crosses reached puberty earlier than CF1s at 8 and 15°C, but when crossed with CF1 males, mice with CF1 dams reached puberty earlier at 22°C (CF1 = 40.5 d; F1 = 48.3 d; CF1 × F1 = 44.41 d). In contrast, ICR maternal crosses reached puberty later than ICRs at 8 and 15°C but earlier at 22°C (ICR = 48.54 d; F1 = 36.30 d; ICR × F1 = 43.75 d). F1s were similar except at 22°C (CF1 × ICR = 48.3 d; ICR × CF1 = 36.30 d). ICR and ICR maternal crosses were consistently heavier at puberty than CF1 counterparts (P < .001). Temperature affected WP (P < .001); mice in the cold environment were heavier (24.36 g) than mice in either 15 or 22°C environment (22.13 and 22.12 g, respectively).

Age at vaginal opening often is used as an indicator of AP. The relationship between these traits was evaluated using a pooled, within-class partial correlation of AVO with AP. The correlation was only .19 (P < .01).

Genetic group affected ovulation rate (OVR) (P < .05) and total fetuses (FETI) (P < .01) but not the total number of implantations (IMP). Backcross genetic groups were consistently higher for all three traits, with F1 genetic groups intermediate. All genetic groups approached 100% implantation of ova.

Temperature affected FETI (P < .01). The lower temperature resulted in fewer FETI (8.6 at 8°C; 10.1 at 15°C and 22°C), with percentage of FETI from implantation (postimplantation survival) decreasing from 93% at 22°C to 83% at 8°C. Percentage of FETI per IMP were similar among crossbred genetic groups (93%) and between pure lines (81%) at all temperatures.
### TABLE 1. ANALYSIS OF VARIANCE FOR FEMALE MOUSE TRAITS

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>df</th>
<th>MS</th>
<th>df</th>
<th>MS</th>
<th>df</th>
<th>MS</th>
<th>df</th>
<th>MS</th>
<th>df</th>
<th>MS</th>
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</thead>
<tbody>
<tr>
<td>Genetic group</td>
<td>5</td>
<td>29.01***</td>
<td>5</td>
<td>2.53**</td>
<td>5</td>
<td>55.70***</td>
<td>5</td>
<td>144.22</td>
<td>5</td>
<td>43.93***</td>
</tr>
<tr>
<td>Temperature</td>
<td>2</td>
<td>3.60</td>
<td>2</td>
<td>0.67</td>
<td>2</td>
<td>2.54</td>
<td>2</td>
<td>3.353.63***</td>
<td>2</td>
<td>113.85***</td>
</tr>
<tr>
<td>Temp x line</td>
<td>10</td>
<td>7.40</td>
<td>10</td>
<td>0.68</td>
<td>10</td>
<td>4.34</td>
<td>10</td>
<td>269.52**</td>
<td>10</td>
<td>7.36</td>
</tr>
<tr>
<td>Regression on entry wt</td>
<td>1</td>
<td>13.90</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Regression on age at weighing</td>
<td>1</td>
<td>31.61**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Residual</td>
<td>155</td>
<td>4.70</td>
<td>188</td>
<td>.87</td>
<td>188</td>
<td>3.58</td>
<td>185</td>
<td>115.87</td>
<td>185</td>
<td>6.49</td>
</tr>
</tbody>
</table>

*P < .05.

**P < .01.

***P < .001.

### TABLE 2. LEAST SQUARES MEANS FOR EIGHT TRAITS OF MICE BY TEMPERATURE AND GENETIC GROUP

<table>
<thead>
<tr>
<th>Traits</th>
<th>8°C</th>
<th>15°C</th>
<th>22°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICR-F1</td>
<td>CF1-F1</td>
<td>ICR-CFI</td>
</tr>
<tr>
<td>42-d wt, g</td>
<td>22.35</td>
<td>20.80</td>
<td>24.85</td>
</tr>
<tr>
<td>Age at vaginal opening, d</td>
<td>27.50</td>
<td>27.00</td>
<td>27.00</td>
</tr>
<tr>
<td>Age at puberty, d</td>
<td>56.16</td>
<td>59.54</td>
<td>48.33</td>
</tr>
</tbody>
</table>
Heterosis was estimated for each trait (Table 3). Heterosis was expressed for WVO and IMP ($P < .05$) and OVR and FETI ($P < .01$) but not for AVO, AP or WP. The four traits with significant heterosis were further analyzed for stability of performance over environments. Regression of each trait on the environmental index provided an estimate of stability for each trait (Figure 1). Although regression coefficients were not significantly different, F1 genetic groups tended to have greater stability of performance (slope closer to zero) across environments for WVO ($b = -.11$) and OVR ($b = .48$). Backcross genetic groups tended to be more stable across environments for IMP ($b = -.59$) and FETI ($b = .008$). Pure lines (0% heterosis) were least stable (greatest slope) for OVR, IMP and FETI but intermediate for WVO.

**Discussion**

Genetic group, but not temperature, influenced weight at 42 d. ICR and ICR maternal crosses were heavier than their CF1 counterparts, suggesting a maternal effect. Although temperature $\times$ genetic group interaction was not significant ($P = .12$), a trend for cold to adversely affect CF1s (20.02, 22.16 and 21.60 g at 8, 15 and 22°C, respectively) but not ICRs (24.85, 23.51 and 24.30 g at 8, 15 and 22°C, respectively) was seen. Temperature effects have been reported in several studies, with wild mice being consistently heavier (Barnett, 1973) and inbred strains being lighter at both weaning (Barnett and Manly, 1956) and as adults (Barnett, 1956) in colder environments; hybrids reared in the cold are heavier than their parents (Barnett, 1965). Hull (1972) found no sire $\times$ environment interaction for weight between F2 crosses of inbred genetic groups compared with F1 crosses.

Heterosis was not significant for 42-d weight, which agrees with results of others (Bandy and Eisen, 1984; Rutledge and Chapman, 1975). Bandy and Eisen (1984) concluded that litter size masked the effect of heterosis for weight measured early in life. Eisen (1973), after selecting mice for 12-d litter weight, found significant heterosis only at 31-d, 42-d, and 70-d weights, supporting Bandy and Eisen’s conclusion. In the present study, heterosis was not significant for 42-d weight despite the fact that we reduced litter size at birth.

Neither temperature nor the G $\times$ E interaction affected AVO or WVO. In contrast, others have reported maturational delay in mice raised in a cold environment. Biggers et al. (1958) reported that 7% of 4-wk-old female offspring of pregnant strain TO mice at 5°C had vaginal introitus, compared with 23% at 21°C. Barnett and Coleman (1959) reported similar findings with three mice strains (A, A2G, C57BL) breeding at -3 and 21°C. Mean age at vaginal opening for their female offspring at -3°C was 33 d, compared with 26 d at 21°C.

Delay in maturation has been correlated positively with small body size; thus, several investigators state that puberty occurs at a critical body weight. Monteiro and Falconer (1966) and Kennedy and Mitra (1963) reported that slow-growing mice and rats, respectively, reach sexual maturity at the same weight but at an older age than mice and rats growing rapidly. Results of the present study do not support this hypothesis. Although genetic group was significant for AVO ($P < .01$), the magnitude of significance of genetic group for WVO ($P < .001$) was greater. Each genetic group, regardless of environment, attained vaginal opening between 26 and 28 d of age;
however, WVO differed substantially among genetic groups. ICRs weighed 15.26, 16.85 and 16.2 g compared with CF1s, which weighed 13.54, 13.14, and 12.96 g at 8, 15 and 22°C, respectively. Others have also reported independence of maturation from body weight in mice (Vandenbergh, 1967, 1969), rats (Orbach and Kling, 1966) and hamsters (Diamond and Yanagimachi, 1970).

Significant heterosis was expressed for WVO but not for AVO. Comparing genetic groups against the environmental index (Figure 1), heterosis reduced phenotypic variation in WVO over environments. The relatively flat line of the F1 compared with other genetic groups (Figure 1) demonstrates F1 homeostasis. However, backcross genetic groups (50% heterozygosity) were less stable (b = 2.2) across environments than purebred genetic groups (b = .77).

Age at puberty was significantly affected by G × E interaction. ICRs reached puberty earlier than CF1s at 8 and 15°C but CF1s reached puberty earlier at 22°C. Little difference in AP for crosses at the three temperatures was seen except that ICR × CF1s reached puberty earlier than all other genetic groups at 22°C. Cold delayed puberty in all genetic groups, an effect frequently observed by others (Biggers et al., 1958; Barnett and Coleman, 1959; Barnett, 1965). Barnett and Coleman (1959) reported that mean age at onset of estrus for mice, determined by vaginal smears, was 38 d at 21°C vs 61 d at -3°C. Our data suggest that "optimum" temperature for age at puberty differs between ICR and CF1 mice.

G × E interaction did not affect WP, although line and temperature, independently, affected (P < .001 for each) this trait. At puberty, mice were heavier at 8°C (24.36 g) than at 15 or 22°C (22.13 and 22.12 g, respectively). CF1 and CF1 maternal crosses
were consistently lighter at puberty than ICR counterparts. Barnett (1965) reviewed influential factors on weight of mice. He found that temperature, as well as protection afforded by nests or presence of other mice, was influential. In our study, faster-growing ICRs reached puberty earlier than slower-growing CF1s. Thus, differences in growth rate counteracted differences in age at sexual maturity; this cancelled the G \times E interaction for WP.

Neither AP nor WP exhibited significant heterosis. Drickamer (1983) reported that heritabilities for AP (vaginal lavage) in a divergent selection experiment were .32 to .58. Heterosis would be less likely to occur for a highly heritable trait.

Puberty is difficult to assess because definitions differ among experiments: vaginal opening, first estrus as determined by vaginal lavage, and first estrus as determined by vaginal plug. Barnett and Coleman (1959) cautioned that puberty (vaginal lavage) was reached only after appearance of “typical cycles.” Vaginal smears containing squamous cells were suggested to be atypical and representative of anovular cycles, not true estrus. Due to these differences in the definition of puberty, comparisons between experiments are difficult and conclusions are elusive. Vaginal opening is an easily identified landmark and often is used to indicate puberty in female mice, although first estrus may not occur until later. It was an objective of the present study to determine the association between these traits. The correlation (.19) between AVO and AP indicates that AVO is poor as an estimator of AP.

Temperature did not affect OVR, a finding supported by Barnett (1973) and Barnett and Munro (1971). However, Barnett suggested that the number of corpora lutea was a poor estimate of OVR because corpora lutea persisted from previous ovulations in cold-adapted mice. Only a single cycle was observed in the present experiment; thus, we cannot comment on Barnett’s observation. However, our data suggest that number of corpora lutea was accurate as an estimator of OVR because implantation was approximately 100% of OVR at all temperatures.

Neither genetic group nor temperature affected IMP. Percentage of implantations of ova approached 100% for all genetic groups, confirming that there was no environmental effect on implantation of ova shed. Barnett (1973) counted fetuses at d 16 of the third gestation. He, too, found no effect of environment on IMP or number of malformed fetuses.

Temperature affected FETI in utero at d 10 of gestation. Pennycuik (1967) found that fertility, defined as carrying a litter full term, declined due to death of offspring earlier and earlier with rising temperature. The cold environment adversely affected FETI (8.63) in the present study, whereas means for FETI were similar in the moderate and control environments (10.07 and 10.12, respectively).

Genetic group affected OVR and FETI, with pure lines performing poorer than crosses. OVR and FETI exhibited strong heterosis ($P < .01$), and IMP displayed moderate heterosis ($P < .05$). Stability parameters were estimated for each trait. As predicted, F1 mice performed more uniformly across environments for OVR ($b = .48$), followed by the backcross ($b = -.71$) and pure ($b = 1.8$) lines. In contrast, backcross mice were, for IMP and FETI, more uniform (stable) compared with the environmental index ($b = -.59$ and $b = .08$, IMP and FETI, respectively). Backcross mice exhibited more heterosis than expected for the three traits, with levels exceeding that of F1s.

Presence of heterosis conferred stability for three of these four traits across environments compared to pure lines. For example, the graphs for pure line and F1 genetic groups for IMP and FETI were consistent in direction of their performance (i.e., decreasing temperature had less of a depressing effect on F1 than on pure lines). Variability across environments was greatest for pure lines, followed by F1s, with backcrosses exhibiting minimal variance.

**Implications**

Animal seedstock production has, through practices such as artificial insemination and embryo transfer, been increasingly concentrated among a few major breeders in recent years. Thus, the ability of the animal to produce consistently across many environments becomes increasingly important. This study suggests that evaluation of stability of performance may be desirable when choosing genotypes for production under uncertain or variable environmental conditions. In addition, these results suggest that crosses are more adaptable and more consistent in performance than are pure lines under variable environments, particularly for the lowly heritable traits.
Literature Cited


