FOETAL AND PLACENTAL SIZE IN A PEROMYSCUS SPECIES CROSS

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Summary. Near-term conceptuses of Peromyscus maniculatus, P. polionotus and their reciprocal hybrids were collected. Foetal weight and crown-rump length were ascertained, as were placental weight and decidual surface area. No significant differences in these measurements were found when the parent species were compared, but the reciprocal hybrids differed significantly from each other and from the parent types in means for these characters. Placentae from reciprocal hybrids showed more than a five-fold difference in weight. They also differed markedly in histological structure. Foetal weight was positively correlated with placental weight in all crosses. These findings are discussed with reference to immunological factors in placentation.

INTRODUCTION

Hybridization of the cricetid species Peromyscus maniculatus, the deermouse, and P. polionotus, the oldfield mouse, is attended by a number of developmental and reproductive anomalies. Reciprocal crosses between the species differ markedly in fertility (Watson, 1942; Liu, 1953a) and F₁ hybrid size (Dawson, 1965). When P. maniculatus is employed as the female parent in the cross, there is no substantial reduction in fertility or litter size but there is a highly significant depression in individual hybrid weight, body length, and other body measurements. On the other hand, if P. polionotus is used as the mother, the cross is much less successful. Few such matings produce litters, and those born usually consist of one or two individuals. Foetal and maternal mortality is common in late gestation. F₁ individuals born to this cross are unusually large—larger than either parent type at equivalent age, and nearly twice the weight of the reciprocal hybrid. These effects are more striking when it is noted that the smaller maternal species, P. polionotus, bears the larger hybrids, and the larger maternal species, P. maniculatus, the smaller hybrids. The relative size effects are evident at birth and persist throughout life.

Male hybrid sterility (Blair & Howard, 1944) and postnatal mortality (Dawson, 1966) are other reproductive disruptions which occur in this cross, but these are not of direct concern in the present report.

The hybrid size effects are also observed in the backcross and F₂ generations; these follow a pattern which assumes a polygenic size inheritance strongly modified by an enhancing or inhibiting maternal effect (Dawson, 1965). The physiological basis of this effect was initially presumed to be hormonal but
several studies have tended to negate this presumption. Among the alternative explanations was the possibility that histocompatibility differences between the species were somehow responsible directly, or indirectly, for both the prenatal mortality and the size phenomenon.

Liu (1953b) suggested that a mechanism analogous to Rh factor in man might produce the prenatal mortality. However, inheritance data (Dawson, 1962) did not support this concept; and the only known erythrocytic antigen differences in _Peromyscus_ did not seem to result in foetal–maternal incompatibility (Rasmussen, 1965). Interest in the antigen hypothesis was revived by studies of strain crosses in the laboratory mouse, _Mus musculus_, performed by Billington (1964) and James (1965, 1967). In these investigations, it was demonstrated that placental hypertrophy occurs when strains differing in histocompatibility factors are crossed. McLaren (1965) and others had established that foetal weight was highly correlated with placental weight in laboratory mouse crosses. In _Peromyscus_, Liu (1953b) had given data based on a small sample (ten placentae) which indicated that F1 hybrids from _P. polionotus_ ⊕ × _P. maniculatus_ ⊖ have larger placentae than the reciprocal hybrids. In view of this finding, it was considered worthwhile to examine further foetal and placental size inheritance in the reciprocal F1 _Peromyscus_ hybrids and to ascertain any dependence of foetal size upon placental size. If there is a dependence, the maternal influence may be histocompatibility factors which act, by analogy with the laboratory mouse, to induce placental hypertrophy and this may result in over-sized hybrids. Since the _Peromyscus_ cross involves two species, they could be expected to differ at many histocompatibility sites.

**MATERIALS AND METHODS**

The stocks of _Peromyscus_ used have been reared in captivity for twenty or more generations and are semi-domesticated. The _P. maniculatus_ stock was derived from animals of the sub-species, _bairdii_, originally collected in Washtenaw County, Michigan. The _P. polionotus_ were originally from Ocala National Forest, Florida, and belong to the race, _subgriseus_. Both stocks breed well in captivity and are routinely maintained by pair matings. Deliberate selection and close inbreeding, especially sib mating, has been avoided; therefore each stock is heterogeneous, but reasonably typical of the wild ancestral populations in physiological and morphological characters.

Healthy, adult mice, aged 4 to 16 months, were used. The four mating classes established for this investigation were _P. maniculatus_ ⊕ × _P. maniculatus_ ⊖ (M), _P. polionotus_ ⊕ × _P. polionotus_ ⊖ (P), _P. maniculatus_ ⊕ × _P. polionotus_ ⊖ (MP), _P. polionotus_ ⊕ × _P. maniculatus_ ⊖ (PM). Thirty to forty continuous pair matings were made for each cross, but not all pairings proved productive.

_Peromyscus_ undergo a _post partum_ oestrus usually within 24 hr after delivery. For the first three classes of matings, females were permitted to deliver and lactate an initial litter. Females pregnant with a second litter were killed on the 23rd day following parturition of the previous litter, and the foetus and placentas were collected at this time. These were, therefore, about 1 day from term. Because of difficulty in obtaining offspring from the fourth cross (PM), a
modified procedure was used to obtain conceptuses. Females, in this class, were examined daily for distension indicating pregnancy. When gravid mice were detected, they were weighed each day subsequently. Since McLaren (1965) had shown that foetal weight in laboratory mice increases by more than 50% in the final 24 hr of gestation, it was assumed that an abrupt gain of 2 g in 1 day in these Peromyscus indicated that term was imminent. Females were killed at once; conceptuses were collected when such a gain occurred. None of the females in the PM cross was lactating.

The procedure for handling and measuring conceptuses applied for all mating categories. Entire uteri were excised and placed in physiological saline; the position of the foetuses in each horn was then recorded. The embryonic membranes were removed from the foetus and gently separated from the placenta, and the placenta was separated from the foetus. Placentae were blotted on filter paper, and the greatest and least diameters were measured with vernier calipers to the nearest millimetre. Placental weights were taken to the nearest hundredth gram. Foetuses were also blotted, crown–rump length measured, and body weight ascertained. Placentae and foetuses were afterwards paired and fixed in formalin–acetic acid solution for preservation or histological study. Throughout the weighing and measuring procedure, care was exercised to prevent excessive dehydration.

Selected placentae representing approximate mean values were embedded in paraffin, sectioned, and stained with eosin–haematoxylin for histological examination.

**Statistical treatment**

Analysis of variance within and between litters of a given mating class in some instances revealed sample heterogeneity. For this reason, tests for differences between mating categories were based upon sibship means as well as upon total individuals. The former, because of the reduced variance, give a conservative probability value. The Student \( t \) test or, where inequality of variance was indicated, a modified \( t \) test was used for detecting differences in mean values between classes. A 0·05 probability was accepted as the level of significance for data based on sibships; and 0·01, for the data based on individuals. Pertinent tests for differences between means were \( M \) to \( P \) (the parental types), \( P \) to MP (the smaller parental type and the smaller hybrid), \( M \) to PM (the larger parental type and the larger hybrid), and MP to PM (the reciprocal hybrids).

Linear regression analysis of placental and foetal weights was performed individually for each litter in all four test classes. Further regression based on the weighted litter means for the four classes was determined, since this measure properly reflects the regression within each mating category. Correlation coefficients were also ascertained for each of the mating classes.

Placental decidual surface areas were calculated from the greatest and least diameters by the approximation:

\[
\pi \frac{d_1 d_2}{4}
\]
RESULTS

The overall fertility of the crosses used in this study was similar to that observed in previous investigations (Liu, 1953a; Dawson, 1965). Of forty established intraspecific P. maniculatus pairs, twenty-seven bore litters with an average number of 3·85 offspring per litter. Thirty-five intraspecific P. polionotus pairs were established; twenty-seven produced an initial litter having a mean of 3·35 offspring. Seventeen of thirty attempted matings between P. maniculatus females and P. polionotus males produced progeny with a mean of 3·06 per litter. In the reciprocal hybrid cross, only four of thirty-five pairings produced an initial litter, with an average of 1·25 newborn per litter. In the last instance, it was conceivable that other pairs may have produced progeny; but because of the procedure used to secure conceptuses, the females generally were killed before term.

Foetal size

The average measurements obtained for foetuses from the four crosses and a summary analysis for mean differences are given in Table 1. There was no detectable difference in any of the foetal measurements when the two species were compared, but highly significant differences were evident in comparisons involving species hybrids. This demonstrated that the size differences observed in previous studies with post-natal animals is also evident in the foetuses.

**Table 1**

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mean Foetal Size of Peromyscus Maniculatus, P. Polionotus and Their Reciprocal Hybrids at Term</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mating type</strong></td>
<td><strong>No. of litters</strong></td>
</tr>
<tr>
<td>P. maniculatus</td>
<td>8</td>
</tr>
<tr>
<td>× P. maniculatus</td>
<td>9</td>
</tr>
<tr>
<td>P. polionotus</td>
<td>6</td>
</tr>
<tr>
<td>× P. polionotus</td>
<td>6</td>
</tr>
</tbody>
</table>

Analysis for mean difference*

<table>
<thead>
<tr>
<th>Test</th>
<th>Weight means</th>
<th>Crown-rump length means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Sibship</td>
<td>Total</td>
</tr>
<tr>
<td>P. maniculatus to P. polionotus</td>
<td>0·3 &gt; P &gt; 0·2</td>
<td>0·4 &gt; P &gt; 0·3</td>
</tr>
<tr>
<td>P. polionotus to P. m.]P. p.</td>
<td>P &lt; 0·0001</td>
<td>P &lt; 0·001</td>
</tr>
<tr>
<td>P. m.]P. p. to P. p.]P. m.</td>
<td>P &lt; 0·0001</td>
<td>P &lt; 0·001</td>
</tr>
<tr>
<td>P. p.]P. m. to P. maniculatus</td>
<td>P &lt; 0·0001</td>
<td>P &lt; 0·001</td>
</tr>
</tbody>
</table>

* Student t probability.
Comparison of foetuses and placentae of *P. maniculatus* (M), *P. polionotus* (P), F₁ hybrids from *P. maniculatus* ♀ crossed to *P. polionotus* ♂ (MP), F₁ hybrids from *P. polionotus* ♀ crossed to *P. maniculatus* ♂ (PM).

(Facing p. 258)
Peromyscus foetal and placental size

The placental size data are presented in Table 2. As in the case of the foetus, there was essentially no difference in size between P. maniculatus and P. polionotus, but marked differences existed between reciprocal hybrids, and between the hybrids and parental types. It is of particular interest that the mean weight of the PM hybrid placenta was more than five times the mean for reciprocal hybrids, while foetuses showed only a 1½-fold difference. It appears that the size influence exerted proportionately greater effect upon the placenta than upon the foetus (Plate 1).

<table>
<thead>
<tr>
<th>Mating type</th>
<th>No. of litters</th>
<th>No. of placentae</th>
<th>Placental wt (g) ± S.E.</th>
<th>Placental area (cm²) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. maniculatus × P. maniculatus</td>
<td>8</td>
<td>37</td>
<td>0.122 ± 0.004</td>
<td>0.122 ± 0.002</td>
</tr>
<tr>
<td>P. polionotus × P. polionotus</td>
<td>9</td>
<td>37</td>
<td>0.117 ± 0.004</td>
<td>0.118 ± 0.001</td>
</tr>
<tr>
<td>P. maniculatus × P. polionotus</td>
<td>6</td>
<td>24</td>
<td>0.057 ± 0.006</td>
<td>0.058 ± 0.002</td>
</tr>
<tr>
<td>P. polionotus × P. maniculatus</td>
<td>6</td>
<td>16</td>
<td>0.344 ± 0.017</td>
<td>0.347 ± 0.024</td>
</tr>
</tbody>
</table>

Analysis for mean differences*

<table>
<thead>
<tr>
<th>Test</th>
<th>Weight means</th>
<th>Area means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Sibship</td>
</tr>
<tr>
<td>P. maniculatus to P. polionotus</td>
<td>0.4 &gt; P &gt; 0.3</td>
<td>0.3 &gt; P &gt; 0.2</td>
</tr>
<tr>
<td>P. polionotus to P. m./P. p.</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>P. m./P. p. to P. p./P. m.</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>P. p./P. m. to P. maniculatus</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
</table>

* Student t probability.

Median and near-median stained sections of six to eight placenta of each type were examined (Plate 2). The histological morphology of the parent types was very similar. In both P. maniculatus and P. polionotus placenta, there was a distinct demarcation between the spongio-trophoblast and labyrinthine

EXPLANATION OF PLATE 2

Fig. 1. Median section of placenta of P. maniculatus.
Fig. 2. Median section of placenta of P. polionotus.
Fig. 3. Median section of placenta of F₁ hybrid from a P. maniculatus ♀ crossed with a P. polionotus ♂.
Fig. 4. Median section of placenta of F₁ hybrid from a P. polionotus ♀ crossed with a P. maniculatus ♂.
D, decidua basalis; ST, spongio-trophoblast; LT, labyrinth. Magnification ×11.
Additional explanation in the text. This plate should be compared with James (1967) opp. p. 270.
layers. The combined decidua and spongio-trophoblast comprised approximately one-third of the placental thickness. The labyrinthine tissue in these was generally homogeneous. The MP hybrid placentae had a much thinner combined spongio-trophoblast and decidual layer, making up one-third to one-fifth of the placental thickness. The spongio-trophoblast was more compact and was clearly delineated from the labyrinthine layer. On the other hand, the PM hybrid had a thick vacuolated spongio-trophoblast which, with the decidua, accounted for one-third to one-half or more of the total placental thickness. The demarcation between the spongio-trophoblast and the labyrinthine tissue was less well defined in the PM hybrid. Much, but not all, of the additional size of the PM placenta is due to more extensive spongio-trophoblast development.

Relationship of foetal and placental size

Text-figure 1 shows linear mean weighted regression of foetal weight on placental weight for each of the classes. In all instances, a strongly positive

![Text-fig. 1. Linear regression of foetal weight on placental weight of Peromyscus maniculatus, P. polionotus and their reciprocal F₁ hybrids based on conceptuses collected within 24 hr of term. Equations are for mean weighted lines. 1, P. maniculatus (y = 0.591 + 6.175x). 2, P. polionotus (y = 0.646 + 5.896x). 3, F₁ P. maniculatus/polionotus (y = 0.725 + 6.116x). 4, F₁ P. polionotus/maniculatus (y = -0.052 + 6.814x).](image-url)
Foetal litters used in this study ranged from two to seven conceptuses with a modal size of four. Regression of litter size on foetal and placental weight was negative in the case of pooled data from all classes and in most instances for each of the individual classes. The difference in mean foetal litter size among the four classes is sufficient to account for only small portions of the disparity in foetal and placental weights shown by the reciprocal hybrid classes, when viewed in reference to the litter size regression. The PM foetuses and placentae are larger and the MP ones smaller than those of the parental classes at any comparable litter size. While the number of liveborn is reduced in the PM cross, the difference between mean litter size at birth and late gestation is accounted for by pre-parturition mortality and resorption (Liu, 1953b).

DISCUSSION

Differences in reciprocal hybrids, presumably alike in average genetic constitution, are generally considered to be indicators of maternal effect. In mammals, such an effect is probably exerted through the egg cytoplasm or through uterine or lactational environmental factors. The pronounced differences in reciprocal hybrids in this *Peromyscus* species cross bring these generalizations into sharp focus. The present study does not clearly resolve the dichotomy of cytoplasmic versus uterine influence on size inheritance, but it does demonstrate that the placentae of these hybrids are subject to this same influence to an even greater extent than the foetuses or post-natal animals. Furthermore, it seems highly likely that foetal size is mediated through the placenta, and that the maternal effect is acting more directly upon the placenta, since the placental response is more pronounced. If the egg cytoplasm were a major component in size determination, a more uniform response of foetal and placental growth might be expected.

The results of this *Peromyscus* study are in some ways analogous to those of inter-strain hybridization in *Mus musculus* obtained by Billington (1964) and James (1965, 1967). In *Mus*, crosses of antigenically different strains produce larger placentae having more extensive trophoblast invasion and thicker decidual layers. However, reciprocal hybrid differences in placental hypertrophy have not been reported. Crosses between *P. polionotus* females and *P. maniculatus* males follow this model. It is more difficult to account for the exceptionally small placentae in the reciprocal cross. These placentae are, on average, smaller than in either parent species. Presumably, if an immunological hypothesis is adopted, a much stronger effect must be exerted in one direction. Unlike *Mus*, the *Peromyscus* stocks are not highly inbred. Consequently, each stock must possess average histocompatibility differences which act upon placental growth in intraspecific crosses to produce normal size placentae. In crosses between *P. maniculatus* females and *P. polionotus* males, there appears to be an immunological tolerance, or histocompatibility factors are only weakly expressed, since the placentae are smaller than in either parent type.

Strong histo-incompatibility between *P. polionotus* females and the foetal PM hybrid could also account for pre-natal mortality in this cross. Liu (1953b) showed that most of the reduction in litter size in this cross is due to foetal death.
and resorption in the latter half of pregnancy and suggested such a mechanism. Perhaps, in many instances, the incompatibility is sufficient to interrupt foetal development.

Two other observations are consistent with the immunological interpretation of differences in this cross. First, Liu (1953b) noted that pre-natal mortality increases with maternal age. This mortality might be expected as antibody titres in the female increased with subsequent pregnancies. Secondly, there is limited evidence that not all crosses between these two species show the reciprocal size and mortality effects. When certain other stocks or sub-species are employed, they appear to be unexpectedly absent. On the other hand, within the species *P. polionotus*, reproductive difficulties of this nature may occur when certain races are hybridized (Bowen, 1968). These results would be expected if there were a few strong histocompatibility factors present in some races or natural populations, but absent in others.

The differentiation of *P. polionotus* and its presumed parent species, *P. maniculatus*, is regarded by evolutionists as a noteworthy case of speciation in progress. The genetic basis and development of partial reproductive isolation are of particular concern. If the immunological hypothesis is correct in this instance, it suggests that histocompatibility factors may function as effective genetic barriers between natural populations of *Peromyscus*.

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