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# The effects of inbreeding on birth weight and foetal and placental growth in mice

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## 1. INTRODUCTION

Although there have been many investigations of the factors affecting birth weight, and its prime component—rate of foetal growth—in mammals, especially mice, we know relatively little about the genetics of birth weight or of its components. Both McCarthy (1965) and McLaren (1965) have reported heterosis in birth weight and in foetal and placental size at late term in crosses of several highly inbred strains of mice. The inference that directional dominance is a property of the genes controlling these traits, in mice at least, might seem warranted. Such a conclusion, however, is at variance with the traditionally held view that birth weight is a character with an intermediate optimum, subject to stabilizing selection and therefore not likely to show directional dominance (for discussion, see Kearsey & Kojima, 1967). Further, the difficulties of extrapolation from the results of crosses between highly inbred lines has been clearly explained by Roberts (1965) in his excellent review of quantitative genetical research on mice. The experiment reported here was undertaken, firstly, to examine the effects of rapid inbreeding on birth weight and prenatal growth in an outbred stock of mice; the detection of inbreeding depression was the criterion for the inference of directional dominance. The second objective of this work was to investigate how inbreeding in the mother and inbreeding in the litter separately affected the rate of prenatal growth. The conclusions refer to only one particular strain; extrapolation is therefore circumscribed, but less so than in any previous experiment because the strain was non-inbred.

## 2. MATERIALS AND METHODS

The base population used in this experiment was bred from an importation of thirty-two mice of an outbred stock, designated the Q-strain at the Institute of Animal Genetics, Edinburgh. The history of these mice is reported in a previous paper (McCarthy, 1967*a*). Twenty-four inbred lines were derived from a cyclically mated base population by mating full-sibs from twenty-four different families. The lines were subsequently propagated by mating the sibs of one litter per line per generation. All lines survived for four generations after which many lines became extinct.

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### (i) *Birth weight*

The effects of inbreeding in four consecutive generations of full-sib mating were estimated by comparing the weighted mean birth weight of all twenty-four lines with the mean of a cyclically mated outbred control population of thirty-two pairs of parents. The numbers of outbred and inbred litters in each generation are shown in Table 1. The number of live young and their collective weight was recorded for the first and second litter of each mating to the nearest decigram on the afternoon after parturition. The mean weight of a litter at birth was the independent variable used in least squares analyses applied to obtain estimates of the effects of inbreeding in each generation.

### (ii) *Foetal weight and placental weight*

Data from which estimates of the effects of different levels of inbreeding in the mother and in the litter on these traits could be obtained were recorded in litters from (*a*) outbred mothers from the control population of Q mice during each of four generations, (*b*) outbred mothers with inbred litters ($F = 25\%$) during the first generation of inbreeding, and (*c*) partially inbred mothers bearing either inbred or crossbred litters during the second, third and fourth generations of inbreeding.

Independent estimates of the effects of four different levels of inbreeding in the litter were obtained in two ways. (1) The effect of raising the inbreeding coefficient of the litter from 0 to 25 % in the first generation of inbreeding was estimated by comparing litters from contemporaneous groups of outbred and sib-mated Q mothers. (2) The effects of accumulated inbreeding in the litter in three later generations of inbreeding were estimated by inference from results obtained on changing the inbreeding coefficient of the litter from 38 %, 50 % or 59 % to 0 % by crossing lines at random; this involved comparison of contemporaneous groups of inbred mothers bearing inbred or crossbred litters.

Independent estimates of the effects of three different levels of inbreeding in the mother ($F = 25\%$, 38 % and 50 %) were obtained by comparing the crossbred litters of groups of partially inbred mothers with the non-inbred litters of contemporaneous groups of outbred mothers from the control population.

Nulliparous pregnant females were dissected on the eighteenth day post-coitum as indicated by the presence of a vaginal plug. On dissection, the uterus was removed and the number of live foetuses in each horn was recorded. The collective weights of the foetuses and placentae were recorded separately for each horn to the nearest centigram. The mean weight of foetuses and the mean weight of placentae in a uterine horn were the independent variables used in least squares analyses applied to obtain estimates of the effects referred to above.

The numbers of pregnant females dissected in the different groups of litters referred to are shown in Table 1. Females from as many inbred lines as possible were included in the experiment; where practicable on crossing lines about half the females of any particular line were outcrossed. Females from 21, 20 and 17 of

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the 24 lines were dissected in the second, third and fourth generations of inbreeding, respectively.

Table 1. *Unadjusted mean values for birth weight, foetal weight and placental weight (± standard errors) for outbred, inbred and crossbred litters in four consecutive generations*

| Generation of experiment | Level of inbreeding (%) | | Birth weight | | Foetal weight | | Placental weight |
|---|---|---|---|---|---|---|---|
| | Mothers | Litters | No. of litters recorded | Mean ± s.e. (g × 10) | No. of ♀♀ dissected | Mean ± s.e. (g × 100) | Mean ± s.e. (g × 1000) |
| 1 | 0 | 0 | 50 | 15·9 ± 0·25 | 58 | 87·5 ± 1·17 | 121·9 ± 1·83 |
| | 0 | 25 | 115 | 15·9 ± 0·18 | 59 | 88·7 ± 0·88 | 125·0 ± 2·12 |
| 2 | 0 | 0 | 52 | 15·7 ± 0·26 | 41 | 87·9 ± 1·23 | 120·2 ± 1·71 |
| | 25 | 38 | 114 | 15·8 ± 0·19 | 61 | 83·6 ± 0·87 | 113·4 ± 1·84 |
| | 25 | 0 | — | — | 61 | 85·7 ± 0·90 | 120·3 ± 1·65 |
| 3 | 0 | 0 | 57 | 14·7 ± 0·25 | 43 | 86·8 ± 1·11 | 112·4 ± 1·63 |
| | 38 | 50 | 96 | 14·7 ± 0·17 | 63 | 81·9 ± 0·96 | 112·2 ± 1·58 |
| | 38 | 0 | — | — | 66 | 85·3 ± 0·79 | 115·6 ± 1·51 |
| 4 | 0 | 0 | 69 | 14·0 ± 0·17 | 59 | 86·0 ± 0·83 | 111·4 ± 1·27 |
| | 50 | 59 | 87 | 14·3 ± 0·16 | 26 | 80·0 ± 2·02 | 116·1 ± 2·27 |
| | 50 | 0 | — | — | 33 | 83·1 ± 2·06 | 122·9 ± 1·97 |

## 3. RESULTS AND DISCUSSION

The unadjusted means of the genetically different groups of litters for the three traits birth weight, foetal weight and placental weight are shown in Table 1 for each of the four generations of the experiment; some of these means will be referred to later. However, for the purpose of interpretation all comparisons were made *within* generations and the means in Table 1 were adjusted for differences between groups in average litter size. Means were adjusted by regression for differences in mean litter size in the case of birth weight and for differences in the mean number of implants in (*a*) the uterine horn and (*b*) the whole litter, in the case of foetal and placental weights. This procedure was adopted from previous experience (McCarthy, 1965) and a summary of the estimates of the effects of the dependent variables on these traits will be published (McCarthy, 1967*b*). It was not necessary to adjust group means for differences in maternal weight; maternal size was not affected by inbreeding in this experiment (McCarthy, 1967*a*).

### (i) *Effects of concomitant inbreeding of mother and litter*

Least-squares estimates of the effects of four consecutive generations of inbreeding on the mean birth weight of young in a litter and on the mean weights of foetuses and placentae in a uterine horn are shown in Table 2. Each estimate is the deviation of the adjusted mean of one group of litters (specified in the previous section and indicated in the table) from the mean of contemporary outbred litters. There was no evidence of inbreeding depression in mean birth weight in any of the four generations of inbreeding. In contrast, inbreeding caused a statistically

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significant reduction in mean foetal weight at 17½ days' gestation in the second, third and fourth generations of inbreeding; the estimates were not inconsistent with a linear decline in foetal size with increase in the concomitant inbreeding of mother and litter. Further, as the observed mean foetal weight in the Q-strain at 17½ days' gestation was of the order of 85 cg, extrapolation to 100 % inbreeding from the estimates in Table 2 suggests that foetal weight could be reduced by at least 10 % of the outbred value.

Table 2. *The effects of 4 generations of inbreeding on birthweight, foetal weight and placental weight*

| Generation of inbreeding | Level of inbreeding ($F$ %) |  | Estimate of effect of inbreeding ± s.e. |  |  |
|---|---|---|---|---|---|
|  | Mothers | Litters | Birth weight (g × 10) | Foetal weight (g × 100) | Placental weight (g × 1000) |
| 1 | 0 | 25 | −0·2 ± 0·30 | −0·6 ± 1·43 | −2·8 ± 2·57 |
| 2 | 25 | 38 | −0·1 ± 0·33 | −4·2 ± 1·46** | −8·5 ± 2·61** |
| 3 | 38 | 50 | 0·0 ± 0·31 | −4·3 ± 1·56** | −3·7 ± 2·37 |
| 4 | 50 | 59 | 0·0 ± 0·26 | −7·6 ± 2·03*** | +3·4 ± 2·73 |

**, ***, Estimate significant at $P = 0{\cdot}01$ and $P = 0{\cdot}001$.

Table 3. *The effects of inbreeding in the litter on foetal weight and placental weight*

| Level of inbreeding ($F$ %) |  | Estimate of effect of inbreeding ± s.e. |  |
|---|---|---|---|
| Mothers | Litters | Foetal weight (g × 100) | Placental weight (g × 1000) |
| 0 | 25 and 0 | −0·6 ± 1·43 | −2·8 ± 2·57 |
| 25 | 38 and 0 | −2·1 ± 1·24 | −6·9 ± 2·39** |
| 38 | 50 and 0 | −3·0 ± 1·21* | −4·7 ± 2·04* |
| 50 | 59 and 0 | −3·0 ± 2·78 | −7·0 ± 2·90* |

*, **, Estimate significant at $P = 0{\cdot}05$, and $P = 0{\cdot}01$.

The estimates of the effects of inbreeding on mean placental weight are more difficult to interpret. While there was a statistically significant depression in the mean value of this trait in the second generation of inbreeding the estimates of the effect of inbreeding in the four generations were not consistent either in sign or magnitude.

### (ii) *The separate effects of inbreeding in the litter and in the mother*

Independent estimates of the effects of inbreeding in the litter on mean foetal weight and mean placental weight are shown in Table 3. Each estimate represents the difference between the adjusted means of two groups of litters specified in the previous section and indicated in the table. All four estimates of the effects of 25 %, 38 %, 50 % and 59 % inbreeding on foetal weight at 17½ days' gestation were negative, but one only was statistically significant; the conclusion that inbreeding depression in foetal weight was due in part to increased homozygosity of the young is, I think, justified. The results obtained with placental weight are even more

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striking; three of four negative estimates were statistically significant. If the validity of the method of evaluating the effects of the litter's inbreeding on this trait is accepted, i.e. by inference from the results of crossing lines at random, then inbreeding in the litter certainly contributed in large part to inbreeding depression in placental weight. This conclusion is consistent with the very striking heterosis in placental weight observed in the $F_1$s between highly inbred lines of mice by Billington (1964), McCarthy (1965) and McLaren (1965). That there is an immunological basis for this heterosis, as suggested by Billington (1964) and James (1965), does not in any way conflict with the interpretation here that placental weight shows inbreeding depression. In the absence of detailed information about the specific alleles of the foetal genotype with immunological effects on the growth of trophoblastic tissue there is, however, no way of deciding whether or not overdominance is involved in the causation of inbreeding depression in placental weight.

Table 4. *Estimates of the effects of inbreeding in the mother on foetal weight and placental weight*

| Level of inbreeding ($F$ %) | | Estimate of effect of inbreeding ± S.E. | |
|---|---|---|---|
| Litters | Mothers | Foetal weight (g × 100) | Placental weight (g × 1000) |
| 0 | 25 and 0 | −2·5 ± 1·46 | −2·0 ± 2·53 |
| 0 | 58 and 0 | −1·4 ± 1·35 | +0·8 ± 2·25 |
| 0 | 50 and 0 | −1·5 ± 2·04 | +8·3 ± 2·50*** |

*** Estimate significant at $P = 0{\cdot}001$.

Independent estimates of the separate effects of inbreeding in the mother are shown in Table 4. Each estimate represents the deviation of the adjusted mean of one group of crossbred litters borne by partially inbred mothers from the mean of a contemporary group of outbred litters. The estimates of the effects of 25 %, 38 % and 50 % inbreeding in the mother on mean foetal weight were negative but not statistically significant. In isolation, these estimates with their large standard errors do not indicate that inbreeding affected the ability of the mother to nurture her young in prenatal life. However, it would appear that the assumption of additive effects of inbreeding in the mother and inbreeding in the litter in the second, third and fourth generations of full-sib mating may be justified by virtue of the apparent additivity of the estimates of the effects of inbreeding in Tables 3 and 4 to yield the highly significant estimates of Table 2.

As intimated earlier, the most difficult aspect of the results to explain were the positive estimates of the effect of inbreeding on mean placental weight in the third and fourth generations of inbreeding. As explained above, the deviation of the mean placental weight of crossbred litters from that of outbred ones was assumed to measure the effects of inbreeding in the mother on placental weight; this approach is satisfactory providing control values do not differ drastically in different generations. While control means *were* consistent for mean foetal weight over the

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whole experiment, mean placental weights for controls were inexplicably low in the third and fourth generations of inbreeding; the means of the controls, shown in Table 1 were 121·9 ± 1·83, 120·2 ± 1·71, 112·4 ± 1·63 and 111·4 ± 1·27 mg in the four generations, respectively. Independent samples from the control population at times different from that of the experiment reported here confirm the inference that the latter two means were abnormally low. Why this should have occurred is not clear. The estimates of the effects of inbreeding in the mother on placental weight, particularly that of 50 % inbreeding, is therefore unrealistic and this is reflected in the last estimate of the effect of inbreeding on this trait in Table 2.

While the results of this experiment provide convincing evidence that inbreeding depression occurs in foetal weight at 17½ days' gestation and less convincing evidence of a similar depression in placental weight, it definitely suggests that mean birth weight does not exhibit inbreeding depression. Taking the results at their face value the inference is: genes controlling the rate of prenatal growth exhibit directional dominance but those controlling birth weight do not. This apparent contradiction can be resolved by implicating some other component of birth weight, not controlled by genes affecting the rate of prenatal growth or affected pleitropically in a compensatory direction by those genes affecting prenatal growth; a trait which falls in the latter class is gestation length. McLaren (1967) summarized the different factors which, in several experiments, had been shown to influence the duration of gestation in the mouse. She concluded: 'In each case, the reduction in gestation period is associated with an increase in the total amount of both foetal and placental tissue.' Further, she concluded from the experiment reported in her paper that it was the mass of the foetus rather than the placenta which determined the gestation length. Turning her statement around, it therefore seems that factors—be they genetic or environmental ones—which decrease foetal mass increase gestation length. The latter was one variable which was not measured in my experiment and for which no statistical adjustment could be made; inbreeding reduced foetal mass and so presumably increased gestation length. Unfortunately, I have no data from which to confirm this inference; it is doubtful, in view of the magnitude of the effects of inbreeding reported here, that significant changes in the duration of gestation would have been detected without large-scale recording of a time-consuming nature. However, the implication that inbreeding affected gestation length in a compensatory way is necessary to explain why birth weight did not show inbreeding depression.

## SUMMARY

Twenty-four lines were bred from a base population of outbred Q mice by continued full-sib mating. Inbreeding did not cause depression in mean birth weight. However, convincing evidence of inbreeding depression in foetal weight at 17½ days' gestation was found; this depression in the rate of prenatal growth was attributable both to inbreeding in the litter and the mother. It was also inferred from the results of crossing the partially inbred lines that inbreeding in

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the litter caused depression of placental weight. It is concluded that the genes controlling the rate of prenatal growth exhibit directional dominance but that the resulting inbreeding depression in this trait does not lead to a lower birth weight, possibly because of the compensating effect of a longer period of gestation.

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