

The effects of inbreeding on the components of litter size in mice

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1. INTRODUCTION

The effects of rapid inbreeding on litter size in one stock of mice (J-strain) were investigated by Falconer and his colleagues (Bowman & Falconer, 1960; Roberts, 1960). They found that the mean litter size declined markedly at the rate of about 0.5 of a mouse per 10% inbreeding and showed, by crossing lines inbred to 50%, that the reduction in litter size was attributable in part to inbreeding in the litter and in part to inbreeding in the mother bearing the litter. Falconer & Roberts (1960) examined the effects of inbreeding in the mother on some component characters of litter size and found that maternal inbreeding increased the incidence of mortality in the preimplantation stages of pregnancy. They found no evidence that ovulation rate was affected by inbreeding and concluded that genes directly affecting ovulation rate in mice did not exhibit directional dominance. In pigs, however, reduction of the ovulation rate had been shown to be almost wholly responsible for the decreased litter size of inbred sows (Squiers, Dickerson & Mayer, 1952; King & Young, 1957). To explain the different findings in mice and pigs, Falconer and Roberts suggested that past selection had acted differently on litter size, and thereby on ovulation rate, in the two species.

Falconer & Roberts (1960) in a limited amount of data detected no suggestion of effects of inbreeding in the litter on the components of litter size; their experiment, however, was not primarily designed to detect such effects. I have since shown by crossing inbred lines that embryonic viability, measured by the incidence of post-implantation mortality, may be affected by inbreeding (McCarthy, 1965). The extent to which changes in this component contribute to depression in litter size in the early generations of inbreeding is not clear.

This paper presents an analysis of the effects of inbreeding on the components of litter size in another stock of mice (Q-strain) which indicates that inbreeding can have different effects on the components of litter size in different stocks of mice and that the responses of the components of litter size to inbreeding cannot be regarded as different in mice and pigs. It also suggests that there is an interaction between levels of inbreeding in the mother and in the litter affecting the incidence of pre-implantation mortality.

2. MATERIALS AND METHODS

The history of the Q-strain was as follows (Falconer, personal communication): one half of the ancestry was from a four-way cross of a strain selected for high litter size, two strains with different origins selected for large body size, and a strain selected for small body size; the other half was from the J-strain unselected control and an inbred strain (JU) derived from this. After its formation the Q-strain was maintained by minimal inbreeding, first for nine generations with ten pair matings per generation, and then for three generations with twenty male and forty female parents.

Twenty-four inbred lines were derived from a cyclically mated outbred population of Q mice 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. In the course of inbreeding, estimates were obtained of the effects of different levels of inbreeding in the mother on (1) ovulation rate, (2) preimplantation mortality and (3) postimplantation mortality. These estimates were obtained by dissecting contemporaneous groups of (a) outbred mothers from the control population of Q mice and (b) partially inbred mothers bearing crossbred litters.

Estimates of the effects of different levels of inbreeding in the litter on pre- and postimplantation mortality were obtained in two ways. First, the effects of raising the inbreeding coefficient of the litter from 0 to 25%, in the first generation of inbreeding, were estimated from results obtained on dissecting contemporaneous groups of outbred and sib-mated Q mothers. Second, the effects of accumulated inbreeding in the litter in later generations were estimated by inference from results obtained on changing the inbreeding coefficient of the litter from 38 or 59% to 0% by crossing lines at random; this involved dissection of contemporaneous groups of inbred mothers bearing inbred or crossbred litters.

Nulliparous pregnant females were dissected on the seventeenth day *post-coitum* and the numbers of corpora lutea, live embryos and dead implants were counted. For each dissection the number of implantations was estimated as the sum of the numbers of live embryos and dead implants and the number of preimplantation losses was estimated as the difference between the number of corpora lutea and the number of implantations. The mortality in each group of litters was expressed as (1) the mean number of losses and (2) the percentage loss in the preimplantation and postimplantation stages of pregnancy. These two measures of mortality estimate, respectively, the average number and the proportion of (a) eggs not represented by implantations and (b) embryos which died at or after implantation. For each group the percentage preimplantation loss was calculated from the ratio of the total number of egg losses to the total number of corpora lutea and the percentage post-implantation loss was calculated from the ratio of the total number of dead implants to the total number of implantations. The statistical significance of the estimates of the effects of inbreeding on (1) the number of losses and (2) the percentage losses was assessed by the following methods:

- (a) the significance of a difference between the mean numbers of losses in two groups of dissections was assessed by a *t*-test or *F*-test, and

(b) the significance of a difference between the percentage losses in two groups was assessed by a χ^2 -test for heterogeneity of the ratios from which the percentage losses were calculated.

Corpora lutea counts presented great difficulty in the Q-strain and derived lines because of the large numbers of eggs ovulated, and also because of the persistence of corpora lutea from the previous ovulation in some ovaries; in the latter case data were not included in analyses. To get a more reliable estimate of the effect of inbreeding in the mother on ovulation rate, egg-counts only were recorded in one generation. Eggs of inbred ($F = 59\%$) and outbred females were counted on the morning after mating. There were no technical difficulties in making these counts.

3. RESULTS

(i) *Litter size at birth and body size*

From the estimates of the effects of inbreeding on the number of live young at birth, shown in Table 1, it is clear that inbreeding depression occurred in the litter

Table 1. *The effects of inbreeding on litter size at birth and six-week weight of females†*

Level of inbreeding ($F\%$)		No. of lines recorded	Litter size		Weight of daughters at six weeks (g.): Deviation from Q \pm s.e.
Mothers	Litters		Mean \pm s.e.	Deviation for Q \pm s.e.	
0	25	24	9.2 \pm 0.29	-0.8 \pm 0.56	-0.4 \pm 0.41
25	38	24	8.8 \pm 0.27	-1.2 \pm 0.49*	-0.9 \pm 0.43*
38	50	24	7.2 \pm 0.27	-2.0 \pm 0.45**	-0.1 \pm 0.39
50	59	24	6.2 \pm 0.27	-3.0 \pm 0.43**	-0.2 \pm 0.35
59	67	18	7.4 \pm 0.33	-2.7 \pm 0.44**	+0.2 \pm 0.42

*, **: Deviation significant at $P = 0.05$, $P = 0.001$.

† Effects of inbreeding estimated as the deviations of the weighted means of lines from the means of contemporary outbred Q controls in this and following table.

size of Q mice. The decrease of litter size on inbreeding was similar in magnitude to that observed when J mice were inbred (Bowman & Falconer, 1960; Roberts, 1960). Also, as found in studies with the J-strain, the average body size of lines did not decline on inbreeding. Estimates of the effect of inbreeding on weights of females only are shown in Table 1 as only these are pertinent to future arguments. The reason for the apparent absence of inbreeding depression in body size, explained convincingly by Roberts (1960), is that the reduction of litter size provided a better preweaning environment for the young and this counteracted any genetic reduction of body size due to the inbreeding.

(ii) *Effects of inbreeding in the mother*

(a) *Ovulation rate*

Estimates of the effects of three levels of inbreeding in the mother ($F = 25\%$, 50% and 59%) on this component of litter size are shown in Table 2. Analyses of data

obtained by counting either corpora lutea or eggs indicated that inbred mothers shed significantly fewer eggs than outbred mothers and that the reduction in ovulation rate was roughly proportional to the level of maternal inbreeding. Since the weight of females was not affected by inbreeding (Table 1), estimates of the effects of levels of inbreeding on ovulation rate in Table 2 were not biased by differences between the weights of inbred and control mothers. For instance, in the comparison of egg-counts (line 3, Table 2) the mean weights of inbred and outbred mothers at dissection were 24.4 ± 0.29 and 24.1 ± 0.34 g., respectively; the difference between these weights was not significant.

Table 2. *The effects of inbreeding in the mother on ovulation rate, number of live young at 17½ days' gestation, numbers of pre- and postimplantation losses, and percentage losses*

Deviation from Q ±s.e.	Level of inbreeding (F%)			
	Litters Mothers	0 25	0 50	0(†) 59
Number of:				
Corpora lutea or eggs		$-1.2 \pm 0.47^*$	$-1.7 \pm 0.48^{**}$	$-2.0 \pm 0.45^{**}$
Live young		$-1.5 \pm 0.62^*$	$-2.4 \pm 0.61^{**}$	—
Preimplant losses		$+0.5 \pm 0.54$	$+1.2 \pm 0.46^*$	—
Postimplant losses		-0.2 ± 0.33	-0.5 ± 0.31	—
Percentage losses: (††)				
Preimplant		$+4.2\%$	$+10.6\%^{***}$	—
Postimplant		$+0.8\%$	-1.9%	—
Sample size:				
No. of lines represented		21	17	15
No. of females dissected		63	37	54

*, **, ***: Deviation significant at $P=0.05$, $P=0.01$, $P=0.001$.

(†) Egg-counts only in this generation.

(††) The significance of a deviation in percentage loss was ascertained by a χ^2 -test for heterogeneity of the ratios, from which this parameter was estimated, in litters of inbred and Q mothers.

(b) Losses and incidence of mortality

Estimates of the effects of two levels of inbreeding in the mother ($F=25\%$ and 50%) on the mean numbers and the percentages of eggs and implants lost in the preimplantation and postimplantation stages of pregnancy, respectively, are shown in Table 2. At the higher level of maternal inbreeding, despite the lower ovulation rate, there were significantly more eggs lost than in litters of outbred Q mothers; the increase in the incidence of preimplantation mortality (estimated by the increase in percentage loss) was therefore highly significant in this case.

In contrast, there was no evidence that the incidence of postimplantation mortality was increased by maternal inbreeding; the percentages of implants lost were almost identical in litters of inbred and outbred mothers as found by Falconer & Roberts (1960).

(iii) *Effects of inbreeding in the litter*

Estimates of the effects of three different levels of inbreeding in the litter on the number of live young at 17½ days' gestation and the numbers of losses in the pre- and postimplantation stages of pregnancy are shown in Table 3. The effects of changing the inbreeding coefficient from 0 to 25% are indicated in the first row of Table 3. *The effects of inbreeding in the litter on number of live young and numbers of pre- and postimplantation losses*

Difference between adjusted means (inbred-noninbred ± s.e.) (†)

Level of inbreeding (F%)		No. of losses		
Mothers	Litters	Live young	Preimplantation	Postimplantation
0	25 and 0	-1.4 ± 0.64*	+1.4 ± 0.58*	-0.1 ± 0.34
25	38 and 0	0.0 ± 0.56	-0.5 ± 0.47	+0.4 ± 0.30
50	59 and 0	-0.4 ± 0.59	-0.2 ± 0.60	+0.5 ± 0.34

*: Difference significant at $P = 0.05$.

(†) Means were adjusted for sampling differences between groups in mean ovulation rate by regressing the numbers of live young and of losses on the number of corpora lutea.

the table; estimates of these effects were obtained by comparing litters of outbred and sib-mated Q females. These estimates were computed as differences between the adjusted means of these two groups of litters; adjustment was made for a non-significant sampling difference in the mean number of corpora lutea between groups using covariance analyses. Changing the inbreeding coefficient of the litter from 0 to 25% resulted in a significant decrease of 1.4 ± 0.64 in the mean number of live young; this decline was attributable to a significant increase in the mean number of preimplantation losses. The total numbers of losses and the corresponding percentage losses in this comparison are shown in Table 4; the highly significant divergence of the ratios of total number of losses to total number of corpora lutea in the two groups of litters bears out the conclusion that the incidence of preimplantation mortality increased in the first generation of inbreeding.

Estimates of the effects of two higher levels of inbreeding in the litter ($F = 38$ and

Table 4. *The incidence of mortality in outbred, inbred and crossbred litters*

Level of inbreeding (F%)		No. of females dissected	Total number of		Preimplant loss (%)	Total number of		Post-implant loss (%)
Mothers	Litters		Corpora lutea	Eggs lost		Implants	embryos	
0	0	54	834	107	12.8*	727	85	11.7
0	25	53	772	155	20.6*	617	78	12.6
25	0	63	880	141	16.0	739	73	9.9
25	38	61	845	103	12.2	742	96	12.9
50	0	37	469	101	21.5	368	37	10.0
50	59	27	331	66	19.9	265	42	15.8

* The two starred percentages differ significantly from each other at $P = 0.001$.

59%), shown in the second and third rows of Table 3, were not consistent with those discussed above. These estimates were inferred from comparisons of inbred and crossbred litters; they were computed, as in the previous case, as differences between means adjusted to a common ovulation rate. Crossing the partially inbred lines did not result in a significant increase in the number of live young at 17½ days' gestation; in consequence, estimates of the effects of the two levels of inbreeding the litter, $F = 38$ and 59%, on the number of live young (Table 3) and measures of mortality (Tables 3 and 4) were not significant.

A comparable anomaly in J-mice was reported by Roberts (1960) with respect to litter size at birth. He found that litter size fell by 1.4 in the first generation of inbreeding as a result of changing the inbreeding coefficient of the young to 25%, but that litter size rose only by a mere 0.5 when the inbreeding coefficient of the young was changed from 50% to 0% by crossing. He implied, in a discussion of this result (Roberts, 1965) that there was an interaction between levels of inbreeding in the mother and litter. The results presented above suggest that the anomaly with respect to litter size, measured as the number of live young at birth by Roberts or as the number of live embryos at late term in this study, is attributable to an interaction between levels of inbreeding in the mother and in the young affecting the incidence of mortality in the preimplantation stages of pregnancy.

4. DISCUSSION

In contrast to the results of Falconer & Roberts (1960) my observations indicate that directional dominance is a property of genes affecting ovulation rate—more correctly, of genes which affect ovulation rate independently of body size—in mice. Available evidence suggests that this is also the case in pigs (King & Young, 1957). The most interesting point, however, is that inbreeding depression in litter size was qualitatively different in two different strains of mice. The occurrence of inbreeding depression in any particular character depends on the segregation in the base population of recessive genes which, in the main, reduce the measure of that character, i.e. which act directionally. The complement of such recessives and their frequencies in any particular strain are determined by its origin and subsequent breeding history. There is, however, no very obvious reason why the Q- and J-strains should differ genetically. Only one-eighth of the whole ancestry of the Q-strain was not also in the ancestry of the J-strain (Falconer, personal communication). The breeding histories of the two strains after their foundation were similar. It is therefore of some interest to note the different effects of inbreeding on ovulation rate in the two strains. This difference suggests that the genes influencing ovulation rate may be rather few in number, since if there were very many genes it would be difficult to account for the difference between two strains with such similar histories.

The two parts of this study should, in theory, have led to a coherent biological explanation of how the concomitant increase in the inbreeding of mother and litter results in an approximately linear decline of litter size during the first three or four generations of inbreeding. This study cannot be said to present such an explanation,

because the results indicate non-additivity of the effects on mortality of inbreeding in the mother and of inbreeding in the litter, which is difficult to interpret in biological terms. Ovulation rate has been discussed in isolation because this component of litter size can be regarded as wholly a character of the mother; the decline in ovulation rate due to inbreeding in the mother was one obvious determinant of the depression in litter size in the second and later generations of inbreeding. The physiological basis of this effect of inbreeding was possibly a reduced level of gonadotrophic hormones in inbred mothers.

From previous work on the crossing of fully inbred lines (Lyon, 1959; McCarthy, 1965) which clearly showed that the incidence of postimplantation mortality only was reduced by crossbreeding in the litter, I had expected that effects of inbreeding in the litter would be explicable in terms of postimplantation mortality. No evidence was obtained that inbreeding in the litter affected the incidence of postimplantation mortality in the first or later generations of inbreeding in Q mice. The results of the second part of this study indicate, however, that the incidence of preimplantation mortality increased in the first generation of inbreeding due to inbreeding in the litter and in later generations due only to inbreeding in the mother. I have pointed out above that the results of Roberts (1960) support the conclusion that the effects of inbreeding in the mother and inbreeding in the young on litter size, and therefore on its components, are not additive. There is no obvious physiological reason why inbreeding in the litter should affect any sub-component of preimplantation mortality, such as the proportion of eggs fertilized or the viability of blastocysts, when the mother is outbred, and not affect that sub-component when the mother is inbred.

SUMMARY

Twenty-four lines were bred from a base population of outbred Q mice by continued full-sib mating. Inbreeding depression in litter size at birth was observed. This decline in litter size was analysed in terms of ovulation rate, the incidence of preimplantation mortality and the incidence of postimplantation mortality. Pregnant females were dissected at $17\frac{1}{2}$ days' gestation and the numbers of corpora lutea, or eggs, and of live and dead embryos were counted. Matings were arranged so that separate estimates of the effects of inbreeding in the mother and in the litter on the components of litter size could be obtained.

In the first generation of inbreeding when the inbreeding coefficient of the litter was raised from 0 to 25% decline in litter size was attributable to an increased incidence of preimplantation mortality.

In the second and fourth generations decline in litter size was attributable to (1) a reduction in the number of eggs ovulated by the inbred mothers, (2) an increased incidence of preimplantation mortality which resulted from inbreeding in the mother. No evidence of significant effects on mortality of inbreeding in the litter was obtained in the later generation of inbreeding.

These findings are discussed in the context of previous work on the effects of inbreeding and crossing on litter size and its components in mice and pigs.

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