THE EFFECT ON LITTER SIZE OF CROSSING INBRED STRAINS OF MICE

J. C. McCARTHY¹

Institute of Animal Genetics, Edinburgh, Scotland

Received September 14, 1964

WHEN females of an inbred strain of mice are mated to males of a different strain, an increase in litter size over the mean of the maternal strain is usually observed. The magnitude of this increase varies. An increase of about 2.2 mice in the mean litter size of BALB/c females was observed on crossing (FORSTHOEFEL 1954; BUTLER 1958). An increase of only 0.2 extra mice was found when C57 females were outcrossed (BUTLER 1958). EATON (1953) recorded increases ranging from 0.3 to 2.5 extra mice on crossing females of nine different inbred strains. The developmental stage at which these increases in litter size occurred was not ascertained. There is evidence, however, that crossing reduces foetal mortality in litters of inbred females. LYON (1959), on dissecting pregnant CBA, C3H and 101 mice, from within-strain and between-strain matings, found that the incidence of postimplantation mortality was significantly reduced by crossing, but that the incidence of preimplantation mortality was unaffected.

The usual genetical explanation of the increase in litter size on crossing inbred females is that it is a manifestation of heterosis in the nonmaternal part of the character, i.e. heterosis exhibited in the young. The implication in this explanation is that the young in inbred litters are homozygous for a number of recessive genes which cumulatively reduce their viability. LYON (1959), however, proposed the alternative explanation that the excess prenatal mortality observed in within-strain matings is due mainly to recessive lethal genes that have recently arisen by mutation and are segregating in the inbred strain. LYON's results, however, did not preclude the more usual explanation in terms of fixed deleterious genes.

This paper describes the effect of crossing inbred strains of mice (1) on litter size at birth and (2) on the potential litter size at different stages of gestation. The results shed some light on the genetical explanation of the effect of crossing on the litter size of inbred mice.

MATERIALS AND METHODS

Litter size at birth: The data on litter size at birth derive from an experiment in which six inbred strains of mice were crossed in all combinations (McCARTHY 1963). As two strains A/Fa and "KL", a local strain, failed to reproduce in the course of this experiment and, therefore, were not used subsequently, the results of crossing are presented for females of four strains only, i.e. CBA/Fa, C57/Fa, R111/Fa and JU/Fa. The last strain is of local origin and is a listed inbred

¹ Present address: Faculty of Agriculture, University College, Glasnevin, Dublin 9, Ireland.

strain (*Standardized Nomenclature for Inbred Strains of Mice* 1960). It had been inbred by full-sib mating for 30 generations when this study began. The CBA, C57 and R111 strains had been maintained in this laboratory by full-sib mating since 1947 when they were originally obtained as inbred stocks. Separate sublines within each strain were not more than five generations removed from a common ancestral pair of full-sibs.

For the purpose of this paper the experiment is best summarized as follows. Nulliparous inbred females were mated singly with full-sib males of the same strain or with males of the five other inbred strains. The numbers of live young in the resulting inbred and crossbred litters were recorded in the first parity.

Litter size at different stages of development: The effect of crossing on litter size at different stages of development was assessed by dissecting pregnant females from within-strain and between-strain matings. The CBA and C57 strains were crossed reciprocally and the R111 and JU strains were crossed reciprocally. These two pairs of reciprocal crosses were chosen because the members of the first pair of strains (CBA and C57) did not differ in litter size while those of the second pair (R111 and JU) differed greatly. This choice of between-strain matings illustrated the range of reciprocal differences in litter size which can occur on crossing inbred strains. The four groups of within-strain matings were set up contemporaneously with the crosses.

For the study of litter size at different stages of development, nulliparous inbred females were mated at eight weeks of age in harems of three females to one male. Females were dissected 17 days after insemination, indicated by the presence of a vaginal plug. Counts were made of (1) the number of corpora lutea, as a measure of the number of eggs shed, (2) the number of live embryos, and (3) the number of dead implants. The sum of (2) and (3) gives the number of blastocysts which implanted at the fifth day of gestation. Dead implants were classified on a morphological basis to indicate the stage of gestation at which death occurred. Those represented by "moles" were scored as "early" deaths. This class, indicating deaths occurring before about the tenth day of gestation comprised the majority of losses. Any implant which was represented at dissection by an undersized placenta without a recognizably formed embryo was scored as a "middle" death. Dead embryos of recognizable form were scored as "late" deaths. The latter two classes were represented by few implants and will be referred to as "later" deaths, which occurred between about the 10th and 17th days of gestation.

RESULTS

Litter size at birth: The mean number of live young at birth in inbred and crossbred litters of each of the four types of inbred female is shown in Table 1. All crossbred litters having the same strain as female parent were pooled, irrespective of the male parent, because it had previously been found (MCCARTHY 1963) that the strain of the male parent did not influence the size of crossbred litters. Crossing significantly increased the litter size of C57 and R111 females by 0.8 and 1.0 mice, respectively, but had a lesser and insignificant effect on the litter size of CBA females. In contrast, crossing did not increase the litter size of the JU females. Crossbred litters of JU females were smaller than inbred litters, though not significantly so.

Litter size at different stages of development: Table 2 shows the number of pregnant females dissected, and the mean number of corpora lutea, implantations, and live young in within-strain and between-strain matings. It is apparent that crossing did not influence the ovulation rate or the number of blastocysts which implanted. Differences between the means of the number of corpora lutea or between the means of the number of implantations for the two groups of females of each strain were not significant, but for one exception. The mean number of corpora lutea in R111 females was significantly greater in between-strain matings

TABLE 1

The number of live young at birth in within-strain and between-strain matings

Maternal strain	Litters	No. of litters	Mean litter size $\pm$ SE	Difference (crossbred minus inbred)
CBA	Inbred	33	5.6 $\pm$ 0.29	0.3 $\pm$ 0.32
	Crossbred	121	5.9 $\pm$ 0.15	
C57	Inbred	21	5.6 $\pm$ 0.33	0.8 $\pm$ 0.35*
	Crossbred	89	6.4 $\pm$ 0.14	
R111	Inbred	23	4.3 $\pm$ 0.34	1.0 $\pm$ 0.45*
	Crossbred	102	5.3 $\pm$ 0.20	
JU	Inbred	33	9.5 $\pm$ 0.33	-0.2 $\pm$ 0.41
	Crossbred	126	9.3 $\pm$ 0.20	

* Significantly different from zero at the .05 level.

than in within-strain matings (at the .05 level). This may be due to sampling error or may be a reflection of real differences in body weight between the two groups of R111 females. No biological significance is given to this anomalous result.

The number of live young at 17 days gestation increased on crossing CBA, C57 and R111 females, but no increase occurred on crossing JU females (Table 2). The observed increases in litter size were not statistically significant. However, the effects of crossing on litter size at birth and at 17 days gestation were of similar magnitude (see Tables 1 and 2). The effect of crossing on the incidence of postimplantation mortality was, therefore, assessed. This is shown in Table 3, where mortality is calculated at the percentage of the total number of implantations, i.e. the proportion of embryos which die subsequent to implantation. The agreement of the two overall ratios of dead to total implants in inbred and cross-

TABLE 2

Mean numbers of corpora lutea, implants and live embryos at 17 days gestation

Maternal strain	Litters	N*	Corpora lutea	Total implants	Live young	Difference in number of live young (crossbred-inbred)
CBA	Inbred	53	7.7 $\pm$ 0.18†	6.8 $\pm$ 0.18	5.8 $\pm$ 0.25	0.5 $\pm$ 0.34
	Crossbred	50	7.4 $\pm$ 0.14	6.9 $\pm$ 0.18	6.3 $\pm$ 0.22	
C57	Inbred	52	8.0 $\pm$ 0.18	7.1 $\pm$ 0.20	6.2 $\pm$ 0.25	0.3 $\pm$ 0.31
	Crossbred	55	7.8 $\pm$ 0.14	7.1 $\pm$ 0.16	6.5 $\pm$ 0.18	
R111	Inbred	58	7.4 $\pm$ 0.13	5.6 $\pm$ 0.25	4.3 $\pm$ 0.23	0.6 $\pm$ 0.35
	Crossbred	61	7.9 $\pm$ 0.14	5.6 $\pm$ 0.28	4.9 $\pm$ 0.26	
JU	Inbred	58	11.0 $\pm$ 0.15	10.0 $\pm$ 0.20	9.3 $\pm$ 0.21	-0.1 $\pm$ 0.30
	Crossbred	54	10.8 $\pm$ 0.18	9.9 $\pm$ 0.23	9.2 $\pm$ 0.22	

* N is the number of females dissected per group.

† $\pm$ Standard errors.

TABLE 3

The incidence of post-implantation death to 17 days' gestation and the distribution of deaths between early (<10 days) and later (10-17 days) stages in inbred and crossbred litters

Maternal strain	Litters	Total No. of implants	Total No. to deaths	Total mortality	No. of early deaths	Early mortality	No. of later deaths	Later mortality
CBA	Inbred	361	55	15.2%*	48	13.3%*	7	1.9%
	Crossbred	344	30	8.7%*	27	7.8%*	3	0.9%
C57	Inbred	370	46	12.4%	43	11.6%	3	0.8%
	Crossbred	389	34	8.7%	33	8.5%	1	0.3%
R111	Inbred	327	79	24.2%**	66	20.2%***	13	4.0%
	Crossbred	345	44	12.8%**	31	9.0%***	13	3.8%
JU	Inbred	584	47	8.1%	38	6.5%	9	1.5%
	Crossbred	532	38	7.1%	29	5.5%	9	1.7%

Ratios represented differ significantly at .05(*), .01(**) and .001(***) levels.

bred litters of each type of female was tested. χ^2 was significant in two cases. This test took no account of heterogeneity between litters in mortality. The latter was tested for by combining the litters in threes, at random, to give a mean expectation of about two dead implants, and calculating the heterogeneity χ^2 for each group of litters. No significant between-litter heterogeneity was found for any group of inbred or crossbred litters (for example, χ^2 for heterogeneity between inbred litters of R111 females, with 19 degrees of freedom was 11.2, which is not significant). Thus, on the basis of the former χ^2 test, significant reductions in postimplantation mortality occurred on crossing CBA and R111 females. The partitioning of this mortality into "early" and "later" is shown in Table 3. About 84 percent of all postimplantation deaths occurred in the early period, i.e., between implantation and the tenth day of gestation. The influence of crossing on the incidence of postimplantation deaths was confined to this early period (Table 3).

None of the inbred strains carried a balanced lethal system. This was apparent from (1) that the total prenatal mortality in any strain did not reach 50 percent, and (2) that the effect of crossing on litter size was small relative to the mean litter size in each case.

DISCUSSION

The results of the two experiments showed good agreement as to the effect of crossing on litter size (see Tables 1 and 2). The increases in litter size at birth and at 17 days gestation in the respective experiments were of similar magnitude on crossing CBA and R111 females, while crossing JU females did not increase litter size in either experiment. The agreement was not as good for C57 females, but the estimates of the effect of crossing are compatible within the limits of sampling error. Crossing influenced litter size by reducing the incidence of post-implantation mortality between the fifth and tenth days of gestation. The reasons

for the large difference in litter size between the reciprocal crosses of the R111 and JU strains—4.3 mice at 17 days gestation—are apparent. Firstly, the strains differed markedly in litter size, secondly the potential litter sizes of the crosses were maternally determined at ovulation and implantation.

The results obtained do not support LYON's (1959) contention that mutant lethal genes contribute appreciably to prenatal mortality in inbred strains of mice. If, as LYON proposed, an appreciable fraction of the postimplantation mortality in within-strain matings is caused by recessive lethals arising by spontaneous mutation, then prenatal mortality should be decreased on crossing *all* inbred strains. This follows from the expectation that the rate of mutation and, therefore, the frequency of lethal homozygotes would be similar in the various inbred strains. This was not found, however. Significant reductions in the incidence of early postimplantation mortality were observed when CBA and R111 females were crossed—the frequency of deaths being reduced by about 50 percent. A lesser reduction was observed on crossing C57 females but in contrast, when JU females were crossed little or no reduction in mortality took place and litter size was not increased by crossing. The conclusion that spontaneous mutant lethals cause extremely little prenatal mortality in inbred strains of mice is supported by the observations of LÜNING (1964) who found no evidence for a repeatable difference in prenatal mortality between matings of related and unrelated individuals of the CBA strain.

The effect on litter size of crossing inbred strains is best explained as heterosis exhibited in the young. The degree of heterosis shown on crossing females of a particular strain reflects the extent to which inbreeding has reduced the viability of the young. In the course of inbreeding, recessive genes which have a deleterious effect on embryonic viability may become fixed in a strain. The number of such genes which become fixed will depend on various factors such as the gene complement of the base population from which the strain is derived, the amount of between-line selection occurring during inbreeding and the rate of inbreeding. It follows that strains are expected to differ from each other in regard to the degree of inbreeding depression suffered in embryonic viability. The breeding history of most inbred strains is obscure, but the JU strain is an exception. It was the only line surviving out of 20 on inbreeding a random-bred population. It showed no decrease in litter size at birth relative to the base population (BOWMAN and FALCONER 1960). Inbreeding depression, therefore, did not occur in either the maternal or nonmaternal components of litter size. The fixation of genes did not result in reduced embryonic viability in this strain. Consequently, crossing has no effect on the litter size of JU females and the prenatal mortality observed in this strain is wholly a character of the mother. The other three strains are homozygous for recessive genes which cumulatively reduce the ability of their embryos to survive through the early postimplantation stage of development. This was most evident in the R111 strain where only 9.0 percent of implantations were lost in crossbred litters at this stage in comparison with 20.2 percent of implantations in inbred litters (Table 3). The conclusions from this work are:

- (1) Increases in litter size observed on crossing inbred strains of mice in this and

other experiments are attributable mainly to the negation of inbreeding depression in embryonic viability; and (2) if mutant recessive lethals do contribute to prenatal mortality in inbred strains, then this contribution is exceedingly small and of little importance in the causation of increase in litter size on crossing inbred strains.

I am indebted to PROFESSOR C. H. WADDINGTON for laboratory facilities, and to the National University of Ireland for financial support. Thanks are due to Drs. D. S. FALCONER and M. F. LYON for helpful comments on the manuscript.

SUMMARY

Females of four inbred strains of mice were crossed. The effects of crossing on litter size at birth and at the different stages in the development of this character were observed. Increases in litter size on crossing three strains were attributable to the effect of crossing at one stage of development i.e. early postimplantation mortality was reduced on crossing. Crossing did not influence the litter size of JU females at any stage of development or at birth. The results are interpreted as evidence for heterosis in embryonic viability. They do not support the hypothesis that mutant recessive lethals cause an appreciable amount of embryonic mortality in inbred strains of mice.

LITERATURE CITED

- BOWMAN, J. C., and D. S. FALCONER, 1960 Inbreeding depression and heterosis of litter size in mice. *Genet. Res.* **1**: 262-274.
- BUTLER, L., 1958 The inheritance of litter size, body weight and variability in a cross between two inbred strains of mice. *Can. J. Zool.* **36**: 969-983.
- EATON, O. N., 1953 Heterosis in the performance of mice. *Genetics* **38**: 609-629.
- FORSTHOEFEL, P. F., 1954 A study of the effect of heterozygosis on litter size in the mouse (*Mus musculus*). *Ohio J. Sci.* **54**: 135-141.
- LÜNING, K. G., 1964 Studies of irradiated mouse populations III. Accumulation of recessive lethals. *Mutation Res.* **1**: 86-98.
- LYON, M. F., 1959 Some evidence concerning the "mutational load" in inbred strains of mice. *Heredity* **13**: 341-352.
- MCCARTHY, J. C., 1963 Analysis of heterosis in mouse crosses. Ph.D. thesis, University of Edinburgh.
- Standardized Nomenclature for Inbred Strains of Mice*. Second Listing—1960. *Cancer Res.* **20**: 145-169.