GENETIC CONTROL OF OVULATION RATE AND EMBRYO SURVIVAL IN MICE.

II. EFFECTS OF CROSSING SELECTED LINES

G. E. BRADFORD AND C. F. G. NOTT

Department of Animal Science, University of California, Davis

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HETEROISIS in characteristics which are part of the reproductive process is a commonly observed result of crossing unrelated stocks in many species (e.g., GOWEN 1952). In mammals, the observed heterosis in litter size could be due to superiority of the crossbred in ovulation rate, in embryo survival, or both. The second of these parameters can be further subdivided into pre- and post-implantation stages. If these components were found to exhibit different degrees of heterosis, the information could be used in developing more efficient animal crossbreeding programs. Such information would also contribute to a better understanding of the nature of the genetic control of litter size.

Analyses by FALCONER and ROBERTS (1960) of crosses of inbred lines of mice indicated that there was directional dominance of genes affecting preimplantation losses but not the other two components. BOSHIER (1968) obtained generally similar results. MCCARTHY (1967) on the other hand, concluded from an inbreeding experiment with a slightly different strain of mice from that used by FALCONER and ROBERTS that genes affecting ovulation rate as well as preimplantation losses showed directional dominance. Again, postimplantation losses appeared to be affected by genes acting additively. In swine, SQUIERS, DICKERSON and MAYER (1952) reported that ovulation rate and embryo mortality, primarily preimplantation but including a small proportion of early postimplantation losses, both showed heterosis or evidence of directional dominance, with ovulation rate showing the greater effect of the two. KING and YOUNG (1957) reached the same conclusion from a comparison of inbred and outbred sows.

In this paper we present the results of crossing lines of mice differing in ovulation rate and in embryo survival, as a result of artificial selection, and we suggest that embryo survival, particularly at the preimplantation stage, exhibits more heterosis than does ovulation rate.

MATERIALS AND METHODS

Three experiments were conducted. General animal management procedures in all three were as described by BRADFORD (1968). All mean litter sizes presented were based on first litters produced by 11-13 week old females.

Experiment 1: Lines selected for ovulation rate (line O) and embryo survival (line E) were crossed reciprocally. The selection procedure and response obtained in these two lines were described in a previous paper (BRADFORD 1969). This experiment was carried out with progeny of

the ninth selected generation of each line. Contemporary unselected control line animals (line C, generation 13) were included in the experiment. Thus there was a total of five groups: O, E, C, O $\times$ E and E $\times$ O crossbreds. The crossbred progeny are designated as F₁'s in the tables. A minimum of 15 litters was produced in each of the five groups.

Mice from these litters were weaned at 21 days. At approximately nine weeks of age, females from each line or cross, either all those available or a random sample, were weighed, mated to males of their own line or cross, and assigned to one of two groups. In the first of these, the animals were sacrificed at approximately 16 (range 14–18) days following the finding of a mating plug. Numbers of corpora lutea (CL) implants (I) and normal fetuses (N) were counted as previously described (BRADFORD 1969), thus providing a measure of ovulation rate (CL), preimplantation loss (CL–I), postimplantation loss (I–N), and litter size (N) for each female. Females with all young resorbing at autopsy (N = 0) were included in the means; these were very few. Females not pregnant at autopsy were excluded, whether or not a mating plug had been found. The animals in the second group were allowed to produce litters.

Experiment 2: Mice from an 8-way-cross line selected for large litter size for 12 generations (line S2, BRADFORD 1968) were crossed reciprocally with a line selected for the same number of generations for rapid body weight gain from 21–42 days (line G). The latter was derived from the same 4-way cross as lines C, E and O described above. In addition to rapid growth rate and large mature body size, line G was characterized by much more variable litter size than line C or any of the other lines in the experiment. Preliminary investigations had suggested that this variability was due to high ovulation rate coupled with large and variable preimplantation losses.

After the F₁ females had been produced, these, plus random samples of the following generation of the parent lines, were mated in a 3 $\times$ 3 factorial design as follows, reciprocal F₁'s being combined as one group (a sample of line C was also mated contemporaneously):

Sire line or cross	Dam line or cross			
	G	S2	F ₁ (G $\times$ S2)	C
	Number of females pregnant/mated			
G	16/20	20/20	22/25	
S2	15/19	18/20	20/23	
G $\times$ S2	17/19	16/20	26/33	
C				25/30

All females from these matings were sacrificed and counts made of CL, I and N at approximately 16 days gestation.

Experiment 3: The results of Experiments 1 and 2 suggested that a more critical measure of the degree of heterosis in the different components would be provided by crossing lines E and G (from Experiments 1 and 2, respectively), since these two lines showed maximum difference, among the stock available, in preimplantation loss, and differed also in ovulation rate and postimplantation loss. Also, both were derived from the same base population. These two lines were therefore crossed reciprocally, using generation 11 and 16 animals from lines E and G, respectively, as parents of the F₁. Progeny from these matings and contemporaneous unselected progeny from the following generation of the parent lines were mated and scored as in Experiments 1 and 2. The mating plan and numbers of females were as follows:

Sire line or cross	Dam line or cross			
	E	G	E $\times$ G	G $\times$ E
E	77/92			
G		31/38		
E $\times$ G			33/37	
G $\times$ E				36/40

Larger numbers of line E females were included since these were the individuals being scored to obtain selection information for the next generation.

Since earlier data had indicated substantially greater variance in several parameters for line G than for the other stocks (a result substantiated in Experiments 2 and 3), the results were analyzed assuming unequal error variances (SNEDECOR and COCHRAN 1968—Chapter 11).

RESULTS

Experiment 1: Results are summarized in Tables 1–3.

Differences in litter size between females producing within-line litters and those producing F_1 litters were not significant, but both line O and line E females had slightly larger crossbred than within-line litters. This suggests a possible small effect of the genotype of the embryo on litter size of the dam.

The difference between line O and line E in litter size (Table 1) is consistent with that regularly observed between these two lines (BRADFORD 1969). The data on prenatal losses (Table 2) also agree with previous observations that embryo survival has declined, relative to the unselected control (C), in line O, and increased in line E. These data are not in particularly good agreement with the distribution of losses in line O between pre- and postimplantation stages shown by the regularly collected data on this line, in that losses at the two stages were about equal in this sample. The more common pattern is for postimplantation losses to be the higher of the two. The ovulation rate in this sample of line O females was also below the average of the line for the preceding few generations.

The average litter size (N) of the F_1 females (Table 2) was not significantly greater than the mean of the parent lines, but since the difference was in the direction and of a magnitude (about 7%) within the range typically found in crosses among unrelated outbred stocks, the assumption is made in the discussion which follows that there was heterosis for number of normal fetuses, i.e., for litter size.

Examination of the different parameters measured reveals that this advantage of the crossbred females was due almost entirely to an advantage at the pre-implantation stage. Losses in the F_1 female at this stage were as low as in the better parent strain. This indicates a strong degree of dominance for low pre-implantation losses, and a consequent heterosis in number of embryos implanted.

TABLE 1

Number born in first litters of females mated within line and to produce F_1 's (Experiment 1)

Sire line*	Dam line*					
	O		E		C	
	Number‡	Mean	Number‡	Mean	Number‡	Mean
O	15/17	8.27	20/20	10.10	...	...
E	16/16	8.69	36/36	9.67	...	...
C	...	...	...	...	34/36	7.65

* C, unselected control; E, selected for embryo survival; O, selected for ovulation rate.

‡ number pregnant/number mated females.

TABLE 3

Litter size of parent line and F_1 females (Experiment 1)

Line	Number mated	Average weight at mating (grams)	Number with litters	Litter size	
				$\bar{X}$	sd
C	36	21.3	32	7.72	2.29
O	18	21.0	18	8.50	2.28
E	36	23.5	36	10.31 (9.75)*	1.58
Average of lines O and E				9.40 (9.12)*	1.93
O $\times$ E	18	21.3	17	9.53	2.32
E $\times$ O	18	21.2	17	9.76	1.86
Average of F_1 's		21.2		9.64	2.06

* Adjusted to a mating weight of 21.25 grams.

Only the average of the reciprocal F_1 's has been considered in the discussion above. The two did differ substantially in I and N, differences which in fact were significant ($P < .05$). This suggests a maternal effect on reproductive performance of crossbred females, since the $O\sigma \times E\varphi$ cross was superior to the reciprocal. However, among those females producing litters (Table 3), the $E\sigma \times O\varphi$ cross had the larger litters. Considering this, the maternal effect postulated seems unlikely. Maternal effect mediated through body weight is, of course, always a possibility in mice (FALCONER 1955). The data in Table 2 indicate no differences in body weight among the groups in this phase of the experiment, eliminating the need to consider its effect, and also suggesting, incidentally, an absence of heterosis in this trait. The data in Table 3 do indicate an advantage in body weight for line E females. The latter were from second litters and were one week older than the other groups when mated.

Litter size of the females allowed to produce litters (Table 3) ranked the different groups similarly to that obtained from N in the autopsied females (Table 2) with the exception of the reversal between the two reciprocal crosses just noted. Adjustment of the line E mean for the difference in body weight noted above, using the regression calculated from these data, increased the F_1 advantage slightly. The generally good agreement between these two groups supports the conclusion that potential litter size can be accurately measured at 16 days gestation, which permits obtaining information on the additional traits, ovulation rate and prenatal losses at different stages.

Experiment 2: Data on litter size in the first phase of the experiment, in which the F_1 litters were produced, are not presented, since a few litters were not counted at birth. Results of the second phase are presented in Table 4 by mating system. Since differences among sire genotypes and sire-by-dam interactions were non-significant for all measures of reproductive performance shown in Table 4, the data are summarized by genotype of dam in Table 5. The two reciprocal F_1 's did not differ from each other, and were also combined.

TABLE 4

Mean numbers of corpora lutea, implants and normals for within-line, F_1 and F_2 matings involving lines selected for litter size (S_2) and gain (G) (Experiment 2)

Sire genotype	Dam genotype																		
	F ₁																		
	S ₂					G					S ₂ × G				G × S ₂				
	Number	CL	I	N		Number	CL	I	N		Number	CL	I	N		Number	CL	I	N
S ₂	18	15.39	13.94	12.94		15	15.13	9.73	8.47		11	16.64	13.64	12.36		9	15.44	13.33	11.67
G	20	15.75	12.45	12.05		16	14.44	10.19	7.94		10	13.70	10.70	9.40		12	13.25	10.58	9.58
F ₁	16	16.31	13.12	12.12		17	16.82	11.24	8.82		8	15.12	12.12	10.88		18	15.33	13.06	12.06

TABLE 5

Ovulation rate, pre- and postimplantation losses of parent line and F_1 females (Experiment 2)

Line or cross	Number of ♀ mated	Ave. wt. (grams)	CL		I		CL-I		N		I-N				
			$\bar{X}$	sd	$\bar{X}$	sd	$\bar{X}$	sd	$\bar{X}$	sd	Percent with zero	Percent with zero			
C	30	20.4	10.96	2.71	10.04	1.88	.92	1.00	44	8.24	2.88	1.80	2.69	36	.79
S ₂	60	22.0	15.80	3.26	13.15	2.90	2.65	2.81	28	12.37	2.46	.78	1.26	46	.78
G	58	28.0	15.50	4.32	10.42	2.99	5.08	5.17	12	8.42	3.26	2.00	2.02	38	.54
Average of															
S ₂ and G ($\bar{P}$)		25.0	15.65		11.78		3.86		20	10.40		1.39		42	.66
F ₁	78	25.1	14.93	3.65	12.29	2.79	2.64	2.66	29	11.09	3.01	1.20	1.23	37	.74
Difference, $\bar{P}$ vs F ₁		n.s.	n.s.		n.s.		P < .05			n.s.		n.s.			

Body weights of the females at mating did, of course, differ in this experiment, since body weight gain was the criterion of selection of one of the parent lines. The mean values for lines S2, G and their F_1 were 22.0, 28.0 and 25.1 grams, respectively (Table 5), which are consistent with those in Experiment 1 in indicating no heterosis in this trait. The regressions of the reproductive variables on mating weight of the female were examined, to determine if adjustment was indicated. These regressions varied markedly between groups. For example, the regressions of N on mating weight were + 0.68, - 0.03 and - 0.03 per gram for S2, G and F_1 females, respectively. Because of this pattern, there seemed no appropriate value for adjusting all groups to a common body weight, and no adjustments for this variable were made. (This was also true for Experiment 3.) It may be noted that the essentially zero regression for line G substantiated a common observation of this line, i.e., that neither the very large nor very small litters were characteristic of females of a particular body size.

Differences among the three dam genotypes, line S2, line G and the F_1 , were not significant for CL, but were highly significant for I, CL-I, N, and I-N, the major factor contributing to this being the much higher losses in line G.

Comparison of the F_1 with the average of the two parents for the three components of litter size, shows the same pattern as Experiment 1. Again, preimplantation losses were equal to the parent with the lower losses, and significantly lower than the midparent average, resulting in an advantage for the F_1 in number of implants.

Experiment 3: The results are presented in Tables 6 and 7.

There were only small, nonsignificant differences in litter size between females producing within-line litters and those producing F_1 litters (Table 6), and thus no evidence to support the suggestion from Experiment 1 that crossbred litters were slightly larger.

Ovulation rate of F_1 females (Table 7) was not significantly different from the midparent average, consistent with Experiments 1 and 2. Preimplantation losses also were not significantly different from the midparent average, in contrast to results of the two previous experiments, though the difference was in the same direction as before, i.e., lower for the F_1 . Postimplantation losses were significantly lower for the F_1 , also in contrast to the results of the earlier experiments. The net effect on litter size was a slightly greater level of heterosis in this experiment (11% *vs.* 7%).

TABLE 6

Number born in first litters of females mated within line and to produce F_1 litters (Experiment 3)

Sire line	Dam line			
	E		G	
	No.‡	Mean	No.‡	Mean
E	30/36	10.63	10/18	8.40
G	13/13	10.08	34/36	8.29

‡ number pregnant/number mated females

TABLE 7
*Ovulation rate and prenatal mortality of unselected and selected lines
 and of F_1 crosses between selected lines (Experiment 3)*

Line or cross	Number of ♀ × ♂ mated	Ave. wt. at mating (grams)	Number pregnant	CL		I		CL-I		N		I-N		N/CL
				$\bar{X}$	sd	$\bar{X}$	sd	$\bar{X}$	sd	$\bar{X}$	sd	$\bar{X}$	sd	
E	92	25.5	77	12.12	1.56	11.74	1.54	.36	.71	10.92	1.81	.82	.94	.90
G	38	34.4	31	14.39	2.82	11.35	3.89	3.03	2.17	8.48	3.84	2.87	2.42	.59
Average of E and G ($\bar{P}$)		30.0		13.25		11.55		1.70		9.70		1.84		.75
G × E	40	28.7	36	13.31	2.07	11.89	2.33	1.42	2.37	10.53	2.68	1.14	1.42	.79
E × G	37	28.5	33	13.67	1.87	12.42	1.30	1.24	1.58	11.03	1.70	1.39	1.22	.81
Average of F_1 's		28.6		13.48	1.97	12.14	1.91	1.33	2.02	10.77	2.26	1.26	1.32	.80
Difference, $\bar{P}$ vs. $\bar{F}_1$				n.s.		n.s.		n.s., $P < 0.2$		$P < 0.025$		$P < 0.05$		

Comparison of the results from Experiments 2 and 3 for line G shows a lower number of corpora lutea and of preimplantation losses, with a lower standard deviation in both, in Experiment 3. This suggests the possibility of overestimation of the number of corpora lutea for some individuals in this line in Experiment 2. The ovaries of some females of this line and its crosses have a different appearance from those of other lines, making the corpora lutea more difficult to count, due apparently to persistence of those from a previous ovulation. Because of this, especial care was taken in counting in Experiment 3, and the counts may be more accurate than in Experiment 2. Recognizing that this could affect the conclusions from Experiment 2, we reexamined the data from all groups in that experiment, and recalculated the means, excluding those in which both ovulation rate and preimplantation loss appeared abnormally high. This recalculation changed the mean values for each group, but did not change the ranking of the groups for any of the parameters, or any of the conclusions drawn. The data are therefore presented as recorded.

Results of all three experiments are summarized in Table 8.

DISCUSSION

The results of the three experiments indicate that there is a moderate degree of heterosis, between 6 and 11% of the midparent average, in litter size of F_1 females. This heterosis is primarily due to lower preimplantation loss, although in one case, lower postimplantation loss contributed significantly to the advantage in litter size.

TABLE 8
Summary of results

Line or cross	CL	I	CL-I	N	I-N
<i>Experiment 1:</i>					
Average of O and E, ($\bar{P}$)	11.13	10.12	1.00	9.06	1.06
Average of F_1 's	11.28	10.76	0.52	9.66	1.10
$(\bar{F}_1/\bar{P}) \times 100$	101.4	106.3	52.0	106.6	103.8
Difference, $\bar{P}$ vs. $\bar{F}_1$	n.s.	n.s.	$P < 0.05$	n.s.	n.s.
<i>Experiment 2:</i>					
Average of S2 and G, ($\bar{P}$)	15.65	11.78	3.86	10.40	1.39
Average of F_1 's	14.93	12.29	2.64	11.09	1.20
$(\bar{F}_1/\bar{P}) \times 100$	95.4	104.3	68.4	106.6	86.3
Difference, $\bar{P}$ vs. $\bar{F}_1$	n.s.	n.s.	$P < 0.05$	n.s.	n.s.
<i>Experiment 3:</i>					
Average of E and G, ($\bar{P}$)	13.25	11.55	1.70	9.70	1.84
Average of F_1 's	13.48	12.14	1.33	10.77	1.26
$(\bar{F}_1/\bar{P}) \times 100$	101.7	105.2	78.5	111.0	68.4
Difference, $\bar{P}$ vs. $\bar{F}_1$	n.s.	n.s.	n.s., $P < 0.2$	$P < 0.025$	$P < 0.05$

The difference between crossbred and parent line average appears to be due to the genotype of the female, rather than that of the zygote, since there was little difference in litter size between parent strain females producing within-line and crossbred litters. There is evidence that litter size of highly inbred females carrying hybrid embryos is larger than that of comparable females carrying inbred embryos (e.g., EATON 1953; LYON 1959; McCARTHY 1965; McLAREN 1965). This effect is, however, usually not a large percentage of the mean, and it is perhaps not surprising that a clear-cut difference was not found in the present experiment involving crosses among non-inbred stocks. The differences between F_1 and parent line dams were of course also smaller in this experiment than typically found when highly homozygous lines are crossed.

With respect to the evidence on mode of action of genes affecting the components of litter size, this appears to be the only study to date from crosses of non-inbred stocks.

The first two experiments provide little evidence on degree of dominance of genes affecting ovulation rate, since the parent lines differed little. The absence of heterosis does provide evidence against overdominance. Experiment 3, where the two lines crossed did differ in this variable, also provides little evidence for dominance. The studies referred to earlier reporting the results of crossing inbred lines (FALCONER and ROBERTS 1960; BOSHIER 1968) all support the conclusion that ovulation rate shows little, if any, heterosis. In fact, one of BOSHIER's two crosses was significantly inferior to the midparent average. In mice, only the inbreeding experiment reported by McCARTHY (1967) shows evidence for dominance of genes affecting this trait. In swine, on the other hand, from results of the two experiments where this has been studied, SQUIERS, DICKERSON and MAYER (1952) and also KING and YOUNG (1957) suggested a considerable degree of dominance for genes affecting ovulation rate.

In all three crosses studied here, crossbred females had lower preimplantation losses than the parental average, the difference being significant in two of the three cases. This pattern is consistent with that found in all of the other experiments referred to above, and there would appear to be sufficient evidence now available to suggest that dominance of genes affecting preimplantation survival is the general rule, at least in mice. This does not, of course, preclude the existence of an additive component of the genetic variance for this variable, and in fact evidence for this is provided by the results of selection in line E (BRADFORD 1969).

The picture is less clear with respect to postimplantation survival. The majority of the crosses studied, including two of three from the present series, indicate additive gene action for this variable. Experiment 3, however, and one cross reported by BOSHIER (1968), show a considerable degree of heterosis. The results of selecting for components of litter size reported earlier from this laboratory (BRADFORD 1969) suggest that it is more difficult to reduce post- than preimplantation losses by selection. This suggests that more heterosis in postimplantation survival might be expected. The evidence to date does not support this idea, but does indicate that heterosis may be found at both stages.

If it were to prove generally true that ovulation rate and postimplantation

survival are traits governed largely by genes acting additively whereas preimplantation survival is strongly heterotic, the information could be used effectively in the exploitation of heterosis for animal production. Crossing strains or inbred lines with similar ovulation rate and postimplantation survival but differing in preimplantation survival should lead to more heterosis than crosses between stocks differing in the first two variables but similar in the last. Further studies, including different species of litter-bearing animals, are needed.

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SUMMARY

Two pairs of selected lines of mice were crossed. Within each pair the two lines had similar ovulation rate but differed in pre- and postimplantation losses. Size of litters appeared to be determined by the genotype of the dam rather than that of the embryos. Crossbred females, however, had larger litters than the midparent average. This difference in both cases was due almost entirely to lower preimplantation losses, ovulation rate and postimplantation losses being very close in each case to the midparent average. From the four lines involved in these two experiments, the pair which showed the greatest difference in preimplantation loss was then crossed. F_1 females from this cross also showed heterosis in litter size due to superior embryo survival, but this was due mainly to lower postimplantation losses.—The results confirm the suggestion from earlier experiments that ovulation rate and embryo survival are controlled by independent genetic systems. They suggest two additional conclusions: 1) pre- and postimplantation survival are also controlled by different genes; 2) genes affecting preimplantation survival exhibit a high degree of dominance, whereas those affecting the other components are more nearly additive. Evidence on the latter point from other experiments with mice and with swine is reviewed; several show the same pattern, whereas some suggest dominance of genes affecting both ovulation rate and postimplantation survival.

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