A STUDY OF THE EFFECT OF HETEROZYGOSIS ON LITTER SIZE IN THE MOUSE *MUS MUSCULUS*

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The experiment reported here was undertaken to test whether litter size in the mouse *Mus musculus* is a trait which is exclusively determined by the mother's heredity and condition, or whether perhaps the heredity of the zygotes, aside from homozygosity for lethal genes, might also have some effect. Since any increase in litter size due to the embryos' heredity might be slight, care was taken to design an experiment with maximum possible sensitivity for this effect. The method used was to compare litter sizes of sister mice from inbred strains one of which was mated to her brother while the other was out-crossed. Appropriate statistical analysis of the data secured showed a significant increase in litter size where the young born were heterozygous. It seems justifiable therefore to conclude that heterozygosity of the young can increase litter size.

The character "litter size" may be defined as the number of young born, whether alive or dead. It is not a simple character but a function of at least three variables, viz., fecundity, number of eggs fertilized, and amount of pre-natal mortality. Fecundity, the number of eggs liberated by the ovaries at an ovulation, naturally sets the upper limit for a possible litter size. The number of eggs ovulated as measured by the number of corpora lutea has been found to increase with parity (the number of times the mother has borne a litter) and maternal age (MacDowell and Lord, 1925; MacDowell, et al., 1929). The increase is rapid from the first to the third litter but slow afterwards, but there is no decline until the eleventh litter.

The other two variables limit the possible litter size. Thus eggs unfertilized and embryos dying before birth will not contribute to the actual litter size attained. Partial or complete sterility in the male naturally will increase the number of eggs unfertilized. The failure of embryos coming to term may be due to various causes. Aside from homozygosity for lethal genes which would not operate in inbred lines, a possible cause could be homozygosity for genes impairing vigor. Under unfavorable conditions in the uterus of a mother in poor health, embryos homozygous for such sublethal genes might fail to complete development. Such genes might be carried by inbred lines. Maternal influences tending to decrease viability of offspring would be such conditions of the mother as would affect her ability to carry and nourish her young. Since litter size regularly decreases in females after the first four or so litters while the number of eggs ovulated does not show a decrease until the eleventh litter, the decrease in litter size is mainly due to an increase in pre-natal mortality (Grüneberg, 1952). Fekete (1947) has found in mice of the DBA strain a high pre-natal mortality (41.7%), and has shown experimentally that this is due to the unfavorable environment of the DBA uterus. An earlier investigation of normal strains which compared the number of living young born with the number of corpora lutea formed during the corresponding pregnancies showed a pre-natal mortality of 33.9 percent (MacDowell, 1924). A change in diet from rolled oats to "Fox Chow" has raised mean litter size from 4.83 ± 0.04 to 5.68 ± 0.04 (Bittner, 1936). Presumably the latter diet increased fecundity or decreased pre-natal mortality or perhaps did both.

Some work which bears on the question whether litter size is determined exclusively by the mother has been reported in the literature. C. V. Green (1931)

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found that litter size was determined wholly by the female in a mouse species cross (*Mus musculus* × *Mus bactrianus*). Wright (1922) in a careful study of the effects of cross-breeding between highly inbred families of guinea pigs also came to the conclusion that the heredity of the young had no effect on litter size. He did not note a slight effect of the heterozygosis of the young in an increase of the percentage born alive. The effect however was insignificant and perhaps due to chance.

EXPERIMENTAL METHODS AND MATERIALS

Description of Mice Used

Two highly inbred strains of mice were used in this experiment, the BALB/c and the DBA/2 strains. The BALB/c mice were descendants of a single pair obtained by E. L. Green from the Jackson Memorial Laboratory in 1937 and were in the 78th to the 79th generations of brother × sister inbreeding. The DBA/2 mice were descendants of one pair of mice received by Dr. Green from Dr. L. W. Law of the National Cancer Institute in 1948 and were in the 36th to 38th generations of brother × sister inbreeding.

The Experimental Design

Originally it was planned to investigate in this experiment the effect of the female and the effect of the male, as well as the effect of the heterozygosity of the young on litter size. Therefore a factorial experiment was designed which incorporated all possible combinations of the experimental material. Let B, B1, B2 be BALB/c strain mice, brother and two sisters, and similarly let D, D1, D2 stand for DBA/2 mice. Four kinds of matings were made: B × B1; B × D2; D × D1; D × B2. The first two matings needed only one cage since the same male was used. For the same reason, one cage sufficed for the last two matings. The young born in each cage from each type of mating were easily distinguished, for the cross-bred young were always brown agouti while the inbred young were always either albino or dilute brown non-agouti. Thus mistakes in classification if made at birth were rectified later when the fur appeared. The four matings described constituted one block. Ten replications (ten blocks) were set up. The animals selected for each block were always approximately the same age and were kept during the experiment under the same environmental conditions. The cages were inspected at least weekly for pregnant mice, which however were not removed from the mating cages. They were left with the males in order that they might mate at the post-partum oestrus and thus have their next litters with less delay. As soon as the young were furred sufficiently to be positively identified as to origin, they were killed. Cages with pregnant females were inspected daily for litters born and the pertinent data recorded. The experiment was begun November 15, 1950, and was terminated on June 6, 1951.

RESULTS OF THE EXPERIMENT

Data Obtained

Matings involving the DBA/2 females produced scanty results. Five of them produced no young at all during the time of the experiment. Since the data secured from the DBA/2 females were incomplete and useless for analysis, they are not given here. The data from the BALB/c females were complete enough for analysis and are given in table 1, where sizes of litter are recorded for each pair of mice compared. Each mouse is identified by her ledger number and in each case the female bred to her own brother is placed first. The litters of the two differently mated sister mice were matched according to date of birth rather than according to the number of the litter in the series. An x marks the absence of one litter of a pair. This method of matching was used in order to make the litters as comparable as possible since mice born at different times might have been carried under somewhat different environmental conditions and the number of eggs ovulated might have differed in mice of different age. Of course it was impossible to match litters exactly as to date of birth; the mean difference between the dates for the matched litters was 5.8 ± 0.68 days. It is possible that some litters were absent because all the young died before birth, or were eaten immediately after birth, or the female failed to conceive for a number of ovulations.

Inspection of these data shows that the matings involving the BALB/c females produced sufficient offspring to allow a reliable statistical analysis. In no case did a BALB/c female produce fewer than two litters.

Results of the Statistical Analysis

As mentioned, the experiment was planned as a factorial experiment, and therefore embodied all possible combinations of the material. Since the matings involving the DBA/2 females produced insufficient results, it was necessary

TABLE 1

Size of litter for BALB/c females. First mouse in pair inbred; second out-crossed. An x means that a matching litter was absent.

MOUSE	LITTER SIZE					
02	5-1*	2	6	3	6	2	x
03	9	11	5	8	6	9	10
09	6	5	(1)#	4	2	6	
10	7	8	9	x	5	x	
36	7	6	8	x			
37	9	10	7	10			
53	1	x	4	4			
54	4	5	8	4			
58	x	12	x	10	3		
57	1	8	8	9	x		
59	2	x	4	x			
60	8	11-1*	6	8			
86	5	2	2				
87	9	x	5				
95	2	4	5				
96	2	9	7				
101	3	6	3				
102	7	(2)#	3				
104	7	9					
105	4	4					

*Refers to the number of mice found dead in the litter.
#Data unusable because of unknown origin or depletion.

to modify the method of statistical analysis originally planned. The analysis of variance with multiple classification of the material was used to test the significance of the differences between litter sizes obtained from the BALB/c females only, as well as to test whether the matched litters for the different pairs of females constituted a homogeneous population. Thus one original object of the experiment was attained, namely, to test for significant differences caused by heterozygosity of the young.

Litter size is considered to be a variable distributed according to the Poisson distribution. This view is based on the idea that the birth of any particular individual mouse is an event whose probability is uniform but ordinarily rather small and that the size of a litter could theoretically at least be quite large. Thus the occurrence of 0, 1, 2, 3, . . . mice in a litter would follow the Poisson distribution, in which the variance is equal to the mean and therefore is perfectly correlated with it. The analysis of variance cannot be used with raw data where there is correlation between means and variances (Bartlett, 1947). A transformation of the data can be used which will eliminate the correlation. The one suitable here is the transformation to $\sqrt{x} + \sqrt{x + 1}$ where x is the raw datum (Freeman and Tukey, 1950). This transformation was performed on the data of this experiment and the statistical analysis was applied to the transformed data. In every case of course only litters which could be matched were used as the source of data. Table 2 gives the transformed data for the litter sizes where the mother was a BALB/c female. The differences between the values for the inbred and outbred mice in each pair is also recorded. The first litter in any pair was the one inbred. A glance at the differences shows a pronounced trend in a positive direction, favoring the litters where the young were heterozygous.

TABLE 2

Litter sizes for BALB/c females transformed to $\sqrt{x} + \sqrt{x+1}$, where x is observed litter size. First mouse in pair inbred; second out-crossed.

MOUSE		PAIRED LITTERS						MEAN DIFFER.
02		4.7	3.1	5.1	3.7	5.1	3.1	
03		6.2	6.8	4.7	5.8	5.1	6.2	
	Diff.	1.5	3.7	−0.4	2.1	0	3.1	1.64
09		5.1	4.7	3.1				
10		5.5	5.8	4.7				
	Diff.	0.4	1.1	1.6				1.02
36		5.5	5.1	5.8				
37		6.2	6.5	5.5				
	Diff.	0.7	1.4	−0.3				0.57
53		2.4	4.2	4.2				
54		4.2	5.8	4.2				
	Diff.	1.8	1.6	0				1.14
58		7.1	6.5					
57		5.8	6.2					
	Diff.	−1.3	−0.3					−0.78
59		3.1	4.2					
60		5.8	5.1					
	Diff.	2.7	0.9					1.77
86		4.7	3.1					
87		6.2	4.7					
	Diff.	1.5	1.6					1.51
95		3.1	4.2	4.7				
96		3.1	6.2	5.5				
	Diff.	0	2.0	0.8				0.91
101		3.7	3.7					
102		5.5	3.7					
	Diff.	1.8	0					0.87
104		5.5	6.2					
105		4.2	4.2					
	Diff.	−1.3	−2.0					−1.58

Once the values for the litter sizes had been transformed and the differences between matched litters calculated, the analysis of variance was used to test whether the differences were actually significant and not merely due to the chances of random sampling. Table 3 summarizes the result of the statistical test. The calculated ratio of the mean squares for "between matings" and "interaction," $F = 12.15$, is greater than the critical value at the 1 percent level of significance, $F = 7.68$. Therefore rejection of the null hypothesis of no difference is indicated.

Once it was established that there was an over-all significant difference in the sizes of the two different kinds of litters, it became of interest to test whether the matched litters for the different pairs of females constituted a homogeneous population. The analysis of variance summarized in table 3 provided information which allowed testing this question. It will be seen that the value of F calculated (the quotient of the mean squares for "between pairs of females" and "between pairs of matched litters from the same female pairs"), 2.75, is about at the critical value for 3.7 percent probability. Therefore there is some reason to think that the litters of the different pairs of females did not constitute an entirely homogeneous population. In the cases of the female pairs 58–57 and 104–105, the inbred sisters had the larger litter sizes. These pairs injected some heterogeneity into the otherwise homogeneous population of litter sizes.

DISCUSSION

This experiment detected a significant increase in litter size which may be ascribable to the heterozygosity of the young. The use of inbred lines enabled the experiment to be quite sensitive to this effect. Litters of a sister inbred differed from the litters of her sister outcrossed only in the fact that the latter contained mice that were heterozygous for at least some loci whereas the former contained mice that were nearly completely homozygous. Thus the significant difference in litter sizes seems ascribable to the single differential factor of heterozygosity.

TABLE 3

Analysis of Variance

Source of Variation	Degrees of Freedom	Mean Square	F
Between matings	1	10.542	12.15
Between pairs of litters	27		
Between pairs of females	9	2.239	2.75
Between matched pairs of litters from same female pairs	18	0.813	
Interaction	27	0.868	

After this experiment was begun, O. N. Eaton in a preliminary report (1950) on extensive experiments made to test for the effect of heterozygosity of dams and litters on characteristics of offspring in mice, reported that greater litter size in certain cases seemed to be due to greater survival among heterozygous mice.

A similar effect on litter size has been found in rabbits. Gregory (1932) observed larger litters from crossbred does than from inbred does. He ascribed this effect to a slightly higher vitality of the hybrid young during the gestation period.

The same effect has been detected in chickens. Byerly (1930) presented evidence which showed that mortality of hybrid chicks in the eggs is less than is the case when the eggs contain embryos resulting from matings between cocks and hens of the same strain.

It has long been known that crosses between different strains often result in a considerable increase in vigor, as shown by size and productivity excelling that of either of the parent strains. This phenomenon of "heterosis" has been exploited by those dealing with hybrid corn. Wright (1922) in his study of the effects of crossbreeding on characters fixed in inbred lines of guineapigs found such heterosis strikingly shown, for example, in increased size of litters from hybrid females, increased resistance to tuberculosis, etc. It is not surprising then that this hybrid vigor can be shown even in utero and that it is manifest in larger numbers of young coming to birth. It is the same phenomenon detected at an early period of development.

Why then did not Green (1931) or Wright (1922) secure evidence for the effect of hybrid vigor operating already in utero? Wright himself said that *a priori* one would expect the inherent vigor of the young in utero to count for something. Litter size is affected by many influences of which the more important known ones have been summarized in the introduction. The effect of hybrid vigor in the young may not be as decisive in influencing litter size as the other factors. Its effect might easily be lost in the random interplay of the other factors. Thus one might expect to detect the effect of hybrid vigor only if the animals used in the experiment are as uniform as possible and subjected to as uniform a treatment as possible except for the one variable whose effect is being tested. It may be that in Green's experiments this ideal was not attained. In the case of Wright's guinea pigs, the experiments extended over four years when environmental conditions were exceptionally varied. He applied corrections for seasonal fluctuations of conditions but perhaps these corrections were not precise enough to avoid loss of the hybrid vigor effect. Another factor may be the small sizes of guinea pig litters. Wright states that litters of from two to four are most common, with litters of more than six quite rare. It is conceivable that hybrid vigor affecting litter size might more easily fail to be detected when the variable has so small a range.

As a conclusion to this study, a possible genetic explanation of the increase in litter size due to heterozygosity will be given. It is modeled on the genetic explanation advanced by Wright (1922) to explain hybrid vigor in general. Dominant deleterious mutations are eliminated rapidly in a species in nature. Various recessive deleterious mutations however can be carried in heterozygous condition and can accumulate. Inbreeding produces homozygosis which is practically complete after the fifteenth generation of brother-sister mating. Thus recessive factors become homozygous and become effective in the inbred strain. Any recessive lethal factors will destroy their possessors but the recessives which are less deleterious can become fixed in the strain and can induce a hereditary lowering of vigor. One sign of this decline in vigor can be failure of some young to complete growth in the uterus and thus fail to be born. Among several inbred strains it is likely that homozygosis of recessive deleterious factors is attained at different loci. Thus crossing between these strains results in an improvement in vigor and litter size because each strain supplies dominant factors lacking in the other which prevent expression of their recessive deleterious alleles.

SUMMARY

A study of the possible effect of heterozygosity of the young or litter size was made. Inbred female mice were bred to their brothers while sisters were outcrossed. Data secured from matched litters of BALB/c mice were sufficient to support a valid statistical analysis. The mean litter size in litters where the young were inbred was 4.82 mice while the mean litter size in litters where the young were hybrids was 6.82 mice. On statistical analysis, the difference of two mice was found to be significant. This difference is ascribable to heterozygosity of offspring which caused greater litter size. The population was not quite uniform in this effect. An explanation is offered of a possible genetic basis for the effect.

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