AN EXAMINATION OF HETEROSIS IN CROSSES OF CERTAIN INBRED STRAINS OF MICE[1]

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The laboratory mouse has many uses in research. Although each particular use may require different characteristics, there has always been a desire for strains of animals with the maximum amount of homogeneity with respect to the characteristics studied. In the ideal strain, every individual would be as much like every other as practicable. Genetic homogeneity has been approached most closely in the development of inbred strains, in which both members of each gene pair are alike. The most common system of development and maintenance of inbred strains in small laboratory animals is through brother-sister mating. Although in the standard general purpose laboratory mouse, the "albino," little attention is paid to genetic uniformity, more than one hundred inbred strains of mice have been developed. The initial purpose of the inbred strains was for cancer research, but now they are recommended for general laboratory use. A further use for inbred strains arose with the realization that the hybrids between two inbred strains are also genetically homogeneous. In the hybrids, although both members of the gene pair may not be alike, all analogous gene pairs in the population are alike. The process of inbreeding may lead to a decline in the vigor of the animals, the amount of decline increasing as the coefficient of inbreeding increases. The crossing of two different inbred strains is partly an attempt to restore a portion of the heterozygosity lost in the inbreeding process. The phenomenon known as hybrid vigor or heterosis may be attributable to the restored heterozygosity.

Numerous articles concerning the general field of heterosis may be found, for example, in a book entitled *Heterosis* edited by Gowen (1952). Concerning specifically mouse breeding, there is an established body of literature on the characteristics of crosses between strains, with some showing evidence of heterosis in the hybrids. Butler (1955), Eaton (1953), and Chai (1960) conclude that heterosis effects are evident in their studies, but Taketomi and Matsuo (1957), Warwick and Lewis (1954), and Bogart et al. (1958) indicate that heterosis is absent in their experiments. The divergence of these findings could be attributable to variation in characteristics measured, in strains used for crossing, and in criteria considered necessary to indicate heterosis.

The purpose of this study is to examine the evidence for heterosis in the crosses between certain inbred strains. The traits reported here are litter size, growth rate, and survival. These are some of the traits in which the decline in vigor of the inbred animal is marked. In this report heterosis was judged present if the measured trait in the offspring from the within-strain matings was significantly lower than that of the offspring from the between-strain matings.

MATERIALS AND METHODS

Animals

The mice used were maintained or produced in the Battelle Memorial Institute colony. They were derived from stocks obtained from the animal colony maintained by Dr. D. W. Bailey at the National Institutes of Health. The strains used and their immediate histories are shown in table 1. Snell et al. (1960) traced the history of these strains. The C3H strain originated with Strong from a cross

[1]The material presented in this paper represents a portion of the thesis submitted by Edwin C. Franks to the Graduate School of The Ohio State University in partial fulfillment of requirements for the degree of Master of Science.

of a Bagg Albino to a DBA; the A/He strain was begun by Strong in 1921, then went to Bittner, 1927, to Heston, 1938, and to Jackson Memorial Laboratory in 1948 at generation 77; the BALB/c's are traced to Bagg in 1906, passed through Little to McDowell in 1922, who then started inbreeding in 1923. The C3Hf strain originated from a litter of C3H born by Caesarean section and fostered on C57BL in 1945. The fostering was done to avoid the mammary tumor factor of the C3H believed transmitted to the offspring through the mother's milk. The strains C3H and C3Hf would be expected, then, to be very similar genetically. The C57BL/10 substrains were separated after 1937 from a strain passed from Lathrop to Little in 1921.

TABLE 1
Inbred strains of mice used in this study

Strain	Obtained from NIH at generation F	Generations used F
C3H	50	50–54
A/He	110	110–113
BALB/c	95	95–98
C3Hf	37	38–40
C57BL/10-H-2^d	29	31–32
C57BL/10Sc	28	28–30

Living Conditions

The laboratory rooms were air conditioned to an approximate constant temperature of 74°F. There was neither humidity control nor day length control from January to September, 1960, the period of the experiment. Stainless steel cages were of standard size. Bedding of cedar shavings was changed weekly. Water from a drinking tube and Purina Mouse Breeder Chow were always available.

Matings

In the experiment being described, the inbred strain C3H was crossed with the inbred strains A/He, BALB/c, C3Hf, C57BL/10-H-2^d, and C57BL/10Sc. Within strain matings and reciprocal between strain matings were made concurrently. All matings were single pair, and once a male and female were placed together, they remained so for the duration of the experiment. This system usually resulted in gestation during lactation and sometimes caused early weaning of the previous litter. A total of 199 litters were used in this experiment.

Collection of Data

When a female was found to be pregnant, she was checked daily for parturition. When a litter was found, the birth date and number of live young were recorded. Beginning with the birth date, day 0, the litter was weighed on days 0, 8, 15, 22, and 30, or as close to those days as possible. If the scheduled weighing day occurred on a weekend, the weighings were done on the nearest weekday and the estimated correct weights interpolated from a previously constructed daily growth curve. The total weight for the litter was determined, and the average weight per mouse found. Immediately following the initial weighing, the litter size was reduced to six mice (with the exception of the first fifty litters).

After the weighing for day 22, the young were weaned and put into a cage separate from their parents, but were not separated according to sex. In a few instances, weaning occurred on day 21 because of the arrival of a new litter from the same parents, but since daily weight curves showed that weaning one day early had no effect on the growth rate, the fact of early weaning was not recorded.

At the time of the final weighing, about day 30, the number of mice of each sex was noted and recorded, thus providing a measure of the survival and of the influence of sex upon the characteristics under study. If a disease tentatively diagnosed as infantile diarrhea were observed in a litter, this also was noted. When the average weight per mouse in the litter reached 12 g, the number surviving was noted.

Statistical Treatment

For the purpose of analysis, the data were arranged into five groups. Group 1 consisted of all the inbred C3H litters, plus all the inbred A/He litters, plus both reciprocal hybrids of these two strains (C3H x A/He, and A/He x C3H). Group 2 consisted of all the inbred C3H litters (identical with that category in group 1), all the inbred BALB/c litters, and both hybrids. Likewise groups 3, 4, and 5 involved inbreds and crosses of C3H with C3Hf, C57BL/10–H–2^d, and C57BL/10Sc respectively. The number of litters in each of the five groups varied from 52 to 79, with the same 26 inbred C3H litters considered in each group.

TABLE 2
List of variables studied

Classification of variable	Variable
Independent	C3H dam
	C3H sire
	C3H both parents
	Parity
	Age of dam (from her birth to parturition)
	Season of birth of litter (weeks, starting January 1)
	Season of birth of litter in weeks squared
	Number born (before intentional limiting, if any)
	Number kept alive past day found
	Proportion females at 30 days
	Infantile diarrhea
Dependent	Average weight day 0
	Average weight day 8
	Average weight day 15
	Average weight day 22
	Average weight day 30
	Proportion survival at 12 grams
	Proportion survival at 30 days
	Number born (before intentional limiting, if any)

A multiple regression analysis was performed with eleven independent variables and eight dependent variables shown in table 2. The model for a multiple regression is

$$Y = b_0 + b_1x_1 + b_2x_2 + \ . \ . \ . \ + b_ix_i$$

where Y is the dependent variable, b_0 is a constant, and the b_i are the partial regression coefficients for each of the independent variables, the x_i. Because the problem was computed as a multiple regression, as any one independent variable was analyzed, all others were adjusted for and held constant. The partial regression coefficient of the third listed independent variable, C3H both parents, was taken as indicative of heterosis if significant and negative.

RESULTS

The partial regression coefficients of the third independent variable, i.e., the effects caused by a litter being inbred rather than hybrid, are listed for each of the dependent variables for each of the five mating groups in table 3. Litter size in the mating groups involving crosses and inbreds of C3H with A/He, BALB/c, C57BL/10–H–2^d, and C57BL/10Sc was significantly depressed by the inbreds. For example, in the first mating group, involving C3H and A/He, the litters that were inbred were born containing, on the average, 2.61 fewer mice than if they had not been inbred. The depression was significant at the .05 level in the crosses involving C3H to A/He and to C57BL/10Sc, and at the .01 level in matings involving C3H with BALB/c and C57BL/10–H–2[d]. The matings involving crosses and inbreds of C3H with C3Hf showed no heterosis; the inbred litters were in fact 1.28 mice larger than the group mean, but this value was not significant.

The weights of the litters were affected adversely by the inbred matings at least during the first thirty days of life. Thus heterosis in growth rate is indicated where these values are negative and significant. In mating groups involving C3H and A/He, C3H and C57BL/10–H–2[d], and C3H and C57BL/10 Sc the

TABLE 3
The multiple regression coefficients indicating the effect of a litter's being inbred instead of hybrid

Variable	Matings of C3H and				
	A/He	BALB/c	C3Hf	C57BL/10–H–2[d]	C57BL/10SC
Litter size (Number of mice)	−2.61*	−3.98**	1.28	−3.53**	−2.57*
Avg. wgt. day 0 (grams)	−0.39**	−0.05	−0.03	−0.51***	−0.26
Avg. wgt. day 8 (grams)	−1.03*	−0.70	1.01	−1.43**	−1.10*
Avg. wgt. day 15 (grams)	−1.22	−0.54	1.50	−1.62*	−1.25
Avg. wgt. day 22 (grams)	−3.95***	−1.29	1.82	−3.91***	−2.94*
Avg. wgt. day 30 (grams)	−4.07*	−2.05	2.35	−4.35**	−4.53**
Survival to 12 g (proportion)	−0.64***	−0.11	0.44**	−0.07	−0.19
Survival to 30 days (proportion)	−0.64***	−0.11	0.44**	−0.07	−0.27

*P<.05, **P<.01, ***P<.001.

depression was generally significant at the .05 or .01 levels. In mating groups involving C3H and BALB/c the inbred depression was not significant; and in the group involving C3H and C3Hf, the inbreds were superior to the hybrids in weight gains, but the superiority was not significant.

The survival of the group involving C3H and A/He was depressed a very significant 64 percent by the inbreds, and the survival in litters from C3H and C3Hf was significantly enhanced by the inbred litters.

TABLE 4
Distribution of groups according to levels of significance of the partial regression coefficients for each combination of dependent and independent variable*

Independent variable	Dependent variable	Negative <.01	<.05	Not significant >.05	<.05	Positive <.01
Dam was C3H	Number born			2,3,4,5		1
	Wgt. day 0			2,3	5	1,4
	Wgt. day 8	3		2		1,4,5
	Wgt. day 15	3		2		1,4,5
	Wgt. day 22			2,3		1,4,5
	Wgt. day 30			2,3		1,4,5
	Survival		3	2,4,5		1
Sire was C3H	Number born			2,3,5	1	4
	Wgt. day 0			2,3,5	1,4	
	Wgt. day 8			1,2,3,5		4
	Wgt. day 15			1,2,3,4,5		
	Wgt. day 22			2,3,4,5		1
	Wgt. day 30			2,3,4	1,5	
	Survival	3		2,4,5		1

TABLE 4—*Continued*

Independent variable	Dependent variable	Negative <.01	<.05	Not significant >.05	<.05	Positive <.01
Increase in parity	Number born			1,2,3,4,5		
	Wgt. day 0				1,2,5	3,4
	Wgt. day 8			2,5	1,3,4	
	Wgt. day 15			1,2,3,4,5		
	Wgt. day 22			2,3,4,5	1	
	Wgt. day 30			1,2,3,4,5		
	Survival			1,2,3,4,5		
Increase in age of dam	Number born		2	1,3,4,5		
	Wgt. day 0			1,2,3,4,5		
	Wgt. day 8		1	2,3,4,5		
	Wgt. day 15			1,2,3,4,5		
	Wgt. day 22			1,2,3,4,5		
	Wgt. day 30			1,2,3,4,5		
	Survival			1,2,3,4,5		
Increase of 1 unit in the season of birth in weeks	Number born			2,3,4,5	1	
	Wgt. day 0		1,3	2,4,5		
	Wgt. day 8		2,3,4	1,5		
	Wgt. day 15		2	1,3,4,5		
	Wgt. day 22			1,2,3,4,5		
	Wgt. day 30			1,2,3,4,5		
	Survival	2,3,4		1,5		
Increase of 1 unit in the square of the season of birth in weeks	Number born		1	2,3,4,5		
	Wgt. day 0			2,4,5	3	1
	Wgt. day 8			1,5	2,3,4	
	Wgt. day 8			1,5	2,3,4	
	Wgt. day 15		1,3,4,5	2		
	Wgt. day 22			1,2,3,4,5		
	Wgt. day 30			1,2,3,4,5		
	Survival			1,5		2,3,4
Increase in number born	Wgt. day 0	2,4,5	3	1		
	Wgt. day 8	3		1,2,4,5		
	Wgt. day 15			1,2,3,4	5	
	Wgt. day 22			1,2,3,4,5		
	Wgt. day 30			1,2,3,4,5		
	Survival			1,2,3,4,5		
Increase in number of mice allowed to live in the litter	Wgt. day 8			5	1,2,3,4	
	Wgt. day 15	2,5	4	1,3		
	Wgt. day 22	2,5		1,3,4		
	Wgt. day 30		5	1,2,3,4		
	Survival			1,2,3,4,5		
Increase in proportion female	Wgt. day 0			1,2,3,4,5		
	Wgt. day 8			1,2,3,4,5		
	Wgt. day 15		3,4	1,2,5		
	Wgt. day 22		2,4	1,3,5		
	Wgt. day 30	4	2	1,3,5		
	Survival			1,2,3,4,5		
Increase in incidence of infantile Diarrhea	Wgt. day 8		4	1,2,3,5		
	Wgt. day 15			1,2,3,4,5		
	Wgt. day 22	2,4		1,3,5		
	Wgt. day 30	2,4	1,3	5		
	Survival			1,2,4,5		3

*Group	Matings involve C3H and
1	A/He
2	BALB/c
3	C3Hf
4	C57BL/10-H-2^d
5	C57BL/10Sc

The effects caused by other independent variables are shown in table 4. The partial regression coefficients of the first independent variable, the strain of the dam, were generally positive, particularly in groups 1, 4, and 5. This means that the average litter would be larger when born, would grow faster, and survive better, if it were from a C3H dam rather than from a dam of another strain. The paternal C3H effects were also positive, but were not so significant as the maternal C3H effects. Each increment increase in parity (the average parity was between 1 and 2, parity being generally synonymous with "litter seriation") significantly increased the weights of the mice on day 0 and day 8. The effects of an increase in the age of the dam were generally negative but not significant. Plotting data of season and season squared developed a parabola with a minimum point. Early in the season the weights at early ages were reduced and the survival rates were depressed. Later in the season these traits were enhanced. The number of mice born in a litter had a significant adverse effect on the weight of each mouse at birth, and the number of mice allowed to live in the litter had adverse effects on subsequent weights. Female mice in groups 2 and 4 were significantly lighter than males at ages of 22 and 30 days. The presence of the disease tentatively diagnosed as infantile diarrhea significantly decreased mouse weights at the age of 30 days in all groups except the fifth, which involved inbreds and hybrids of C3H and C57BL/10Sc.

TABLE 5

Square of the multiple correlation coefficient

	Matings of C3H and				
Dependent variable	A/He	BALB/c	C3Hf	C57BL/10-H-2^d	C57BL/10Sc
Litter size (proportion)	0.273**	0.260*	0.247	0.266*	0.202
Wgt. day 0 (proportion)	0.326**	0.287*	0.453**	0.525**	0.357**
Wgt. day 8 (proportion)	0.417**	0.562**	0.657**	0.623**	0.474**
Wgt. day 15 (proportion)	0.357**	0.687**	0.591**	0.538**	0.656**
Wgt. day 22 (proportion)	0.374**	0.498**	0.448*	0.616**	0.437**
Wgt. day 30 (proportion)	0.224	0.545**	0.410*	0.513**	0.380*
Surv. 12 g (proportion)	0.483**	0.348*	0.491**	0.338*	0.150
Surv. 30 day (proportion)	0.483**	0.348*	0.491**	0.338*	0.187

*P<.05, **P<.01

The square of the multiple correlation coefficient, denoted R^2, indicates the proportion of the total variability of a dependent variable accounted for by the considered independent variables. The R^2 values for this experiment are shown in table 5. The level of significance is from the "F" table which tests the reliability of the difference of the values from zero; significance indicates that a real portion of the variability is attributable to the analyzed independent variables. From table 5 it can be seen that the variation accounted for by the independent variables considered ranged from 15.0 percent to 68.7 percent. Relative to the data on weights at various ages wherein highly significant squares of the multiple correlation coefficient were found, the considered variables were important in bringing about variation in these characteristics. In regard to the two measures of survival that were used the squares of the multiple correlation coefficients were of a lower magnitude than in the measures of weight. Thus the independent variables taken into account were less important in causing variation in survival than in weights. Litter size was in general even less affected than survival by the considered variables as is indicated by the lower R^2 values, two of which did not reach a magnitude great enough to be significant.

DISCUSSION

As can be seen from table 3, heterosis was found to be present in litter size, growth rate, and survival in hybrids of C3H and A/He. Heterosis was also present in litter size and growth rate in hybrids between C3H and the two C57BL/10 substrains. In hybrids derived from mating C3H and BALB/c, heterosis was present only in litter size. Growth rate and survival of the C3H-BALB/c hybrids showed the inbreds to be inferior, but not significantly so.

The hybrids of matings of C3H to C3Hf were inferior to the inbreds, not significantly, but still in a direction opposite to that which would indicate heterosis. Although the positive values indicated for litter size and growth rate were not significant, they become noteworthy when it is seen that all other terms for litter size and growth rate on table 3 are negative. Realizing that the strains C3H and C3Hf are genotypically similar, one would not expect to find any significant differences between hybrids and inbreds in these strains, and indeed, this was true for all the litter measurements except survival.

Upon crossing the strains D and sc, Taketomi and Matsuo (1957) found the mortality of the F_1's up to 20 days of age to be intermediate between the parental strains. They found also that the F_1 mice arrived in smaller litters than did the inbreds, but grew faster up to 20 days of age, possibly because of having fewer mice to share the dam's milk supply.

Warwick and Lewis (1954) [defining heterosis in terms of "overdominance," i.e., to be heterotic the hybrids must exceed the highest parent] found that in crossing large and small strains of mice, the hybrids did not exceed the body size of the larger parent, and hence concluded that there was not heterosis in this trait.

The work of Bogart et al. (1958) demonstrated the hybrids from various combinations of four strains of mice to be generally hyperthyroid, hence surviving asphyxiation for shorter periods of time. The growth rate of the F_1's was shown to be inferior to the growth rate of the parents. Some of the strains produced the most mice per litter when crossed to other strains, but other strains produced more when inbred.

In no case were these experimenters who demonstrated the lack of heterosis dealing with the identical strains of mice as were used in the present experiment or as used in the work of Butler (1955), Eaton (1953), or Chai (1960). Besides the variation in the traits studied, there was also variation in the criteria for determining the trait, e.g., the measurement of survival under asphyxiation rather than in the more common sense of under the stress of normal laboratory factors. A third variant between experimenters was that some required a mouse to be superior to both parents in order to be classed as showing heterosis, and others, as in the present experiment, defined the heterotic hybrid as that which was superior (significantly) to the mid-parent value.

SUMMARY AND CONCLUSIONS

An experiment was designed to determine whether or not heterosis was present in crosses of certain inbred strains of mice. The strain C3H was crossed with strains A/He, BALB/c, C3Hf, C57BL/10-H-2^d, and C57BL/10Sc. Both reciprocal hybrid types were compared with the mid-parent value as a measure of heterosis in litter size, weight per mouse in the litter at the age in days of 0, 8, 15, 22, and 30, and survival to 12 g of weight and to 30 days of age. A multiple regression analysis determined the effects of many independent variables on the dependent variables.

With one exception, heterosis in litter size was present in the five groups; the hybrid litter size in three of the five groups was superior to that of either parent strain. Heterosis in birth weight was significant in two of the groups. In growth

rate, measured in terms of weight per age, heterosis was evident in three groups at day 8, in one at day 15, three at day 22, and three at day 30. Heterosis in survival was evident in only one group of the five. Hybrids between C3H and C3Hf were inferior to the inbreds in litter size, in all weights except birth weight, and in survival.

ACKNOWLEDGMENTS

Appreciation is expressed to Dr. Ransom Whitney and The Ohio State University Research Center Numerical Computation Laboratory for aid in the statistical arrangement and solution of the problems. Thanks are extended to Dr. William McIntosh for suggestions in certain phases of the research, and to Robert Tissot for assistance in the care of the mice and associated laboratory duties. Laboratory facilities were provided by the Cancer Chemotherapy National Service Center through Battelle Memorial Institute.

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