HETEROSIS IN CROSSES OF INBRED LINES OF RATS[1]

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HETEROSIS has been the subject of intense theoretical and applied research for more than 50 years, largely because of its important implication for both evolution and breeding practice. Among the mammals heterosis has been observed as a result of crossing breeds, strains or inbred lines of cattle, sheep, swine, rabbits, guinea pigs, rats, and mice. A detailed review of the vast literature compilation concerning heterosis in mammals is neither possible nor desirable in this paper. Reference is made only to those papers relevant to this experiment.

The theoretical aspects of the problem of specific and general combining ability in swine have been investigated by HENDERSON (1948, 1949, 1952). DICKERSON (1952) considered the use of inbred lines for heterosis tests with particular reference to swine, and presented an interpretation of the evidence concerning the sort of genetic system underlying economically important traits. He also compared the effectiveness of several alternative breeding methods.

The experiment described herein was devised to study the degree of heterosis, general and specific combining ability, sex linkage, and maternal influence arising from crosses among four inbred lines of rats. The design and analysis was largely predicated on the cited work of HENDERSON and DICKERSON. The data refer to 28-day (weaning) and 70-day body weight.

MATERIALS AND METHODS

Four inbred lines were available for this investigation. An albino line, designated B, was obtained from the Department of Genetics, University of California, Berkeley. An albino and a black line were obtained from the Department of Animal Husbandry, University of California, Davis, and are referred to as A and D, respectively. The fourth line, designated H, was black hooded, and was developed in this laboratory from a commercial stock. Inbreeding coefficients were not exactly determined, but were estimated to average above 60 percent in

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all lines and approached 90 percent in some, the Davis rats being the most highly inbred and the hooded rats the least.

A standard complete ration developed in this laboratory was fed to all animals. Females were first mated after 100 days of age. First litters only were used in this study. Litters were reduced to six at five days. An attempt was made to retain three males and three females, but no selection was practiced within sexes. Litters were weaned and weighed at 28 days. The rats were fed in like-sexed groups of three or six, depending on cage size, from 28 through 70 days when a final weight was taken. A single unshrunk weight was used. No artificial selection with respect to body size had been applied to the lines used in this study.

All possible crosses, including linebreds and reciprocals, were made among the four lines. At least four litters were produced in each of the 16 categories with a total of 104 litters. Litter averages for each sex rather than individual weights were used in the analysis. Hence, there were 208 observations each for 28- and 70-day weight.

The mathematical model used in the analysis was an extension of the one formulated by HENDERSON (1948) to include the effects of sex, heterosis, and linebreds as follows:

$$y_{ijklm} = \mu + s_i + h_j + p_{1k} + g_{2k} + g_{2l} + d_{2kl} + r_{2kl} + m_{2l} + e_{ijklm}$$

where:

y_{ijklm} = the average weight (28- or 70-day) for the mth litter in the cross (or linebred) obtained by crossing the kth line of sire with the lth line of dam and for the ith sex. The j subscript identifies whether the result obtained is an inbred line ($j = 1$) or a cross ($j = 2$)

μ = the general mean when equal numbers exist with respect to all effects in the model

s_i = the effect of the ith sex

h_j = the effect of the jth type of cross, i.e., linebred or cross

p_{1k} = the kth linebred within the linebreds

g_{2k} = the general combining ability effect from the kth line of sire

g_{2l} = the general combining ability effect from the lth line of dam

d_{2kl} = the specific combining ability for the kl or lk cross

r_{2kl} = the sex-linked effect for the kth line of sire mated to the lth line of dam

m_{2l} = the maternal effect for the lth line of dam measured over the crosses only. In fact, the g's, d's, r's, and m's are measured from the crosses only

e_{ijklm} = an effect common to all sex-litter subclasses

All constants were obtained by a conventional least squares analysis and all tests of significance were made under the assumption that all effects (except the set being tested) did exist.

RESULTS AND DISCUSSION

The analysis of variance of 28- and 70-day weights is presented in Table 1. The least squares means for the various comparisons are presented in Table 2. Since specific comparisons would be of extremely limited interest, a test of significance

TABLE 1

Analysis of variance in 28- and 70-day weights (mean squares only)[1]

Source of variation	d.f.	Mean squares	
		28-day weight	70-day weight
Sexes (S)	1	543.4**	204,992**
Heterosis (H)	1	211.6	2,777*
Linebreds (L)	3	628.9**	9,146**
General combining abilities (G)	3	353.4**	1,114
Specific combining abilities (D)	2	90.2	241
Sex linkage effects (R)	3	89.4	907
Maternal effects (M)	3	577.7**	4,425**
Error	191	70.5	655

[1] Adjusted for unequal frequencies by least squares.
* Significant at .05 level of probability.
** Significant at .01 level of probability.

TABLE 2

Least squares means

Classification	No.	28-day weight	70-day weight
Sexes $(\hat{\mu}+\hat{s}_i)$			
Males	104	61.9	218.3
Females	104	58.7	155.5
Over-all $(\hat{\mu})$	208	60.3	186.9
Heterosis $(\hat{\mu}+\hat{h}_j)$			
Linebreds	72	59.2	182.9
Crosses	136	61.4	190.9
Linebreds $(\hat{\mu}+\hat{h}_1+\hat{p}_{1k})$			
AA	22	55.9	175.8
BB	26	66.3	213.8
DD	12	53.7	170.3
HH	12	60.9	171.7
Genic breeding value for crossing $(\hat{\mu}+\hat{h}_2+2\hat{g}_{2k})$			
A	72	67.4	206.1
B	64	58.6	194.3
D	68	67.0	185.9
H	68	52.6	177.3
Maternal effects $(\hat{\mu}+\hat{h}_2+\hat{m}_{2l})$			
A	38	59.7	181.1
B	32	62.9	211.7
D	32	55.6	182.1
H	34	67.4	188.7

Classification	No.	28-day weight	70-day weight
Crosses $(\hat{\mu}+\hat{h}_2+\hat{g}_{2k}+\hat{g}_{2l}+\hat{d}_{2kl}+\frac{\hat{m}_{2k}+\hat{m}_{2l}}{2})$			
AB	24	61.6	203.9
AD	22	63.3	186.0
AH	26	63.7	188.2
BD	22	62.1	198.6
BH	18	59.1	194.4
DH	24	58.6	174.3
Reciprocal crosses $(\hat{\mu}+\hat{h}_2+\hat{g}_{2k}+\hat{g}_{2l}+\hat{d}_{2kl}+\hat{r}_{2kl}+\hat{m}_{2l})$			
AB	12	59.2	184.1
AD	12	64.3	182.7
AH	14	61.6	191.7
BA	12	64.0	223.7
BD	12	64.6	211.5
BH	8	57.3	203.3
DA	10	62.2	189.3
DB	10	59.7	185.7
DH	12	50.5	166.3
HA	12	65.7	184.7
HB	10	61.0	185.5
HD	12	66.7	182.3

of differences among the means such as that described by DUNCAN (1955) and extended by KRAMER (1957) was not made.

The highly significant difference between sexes in favor of the males is in agreement with previous findings (KING 1915; LIVESAY 1930; BECKER 1957).

Heterosis was measured as the comparison of inbreds with line crosses. A more desirable comparison would have been between line crosses and the outbred populations from which the inbred lines were derived. This comparison would indicate whether the superiority of the line crosses was merely the recovery from inbreeding degeneration or a true superiority. Heterosis was not quite significant at 28 days but was significant at 70 days. LIVESAY (1930) made two crosses among three inbred lines of rats and observed heterosis for body weight at 30, 50, 70, 90, and 150 days. The relative superiority of the line crosses increased with increasing age. In the present data, maternal effects may have been relatively greater at 28 days and tended to mask the effect of heterosis. It is also possible that heterosis affects general postnatal growth rate and that differences become more pronounced with increasing age. DICKERSON, LUSH, and CULBERTSON (1946) compared single-cross with inbred litters by the same sire among 11 inbred lines of swine and found that with respect to weight the line crosses tended to exceed the inbreds to a greater extent with increasing age.

Estimates of the heterosis achieved in each cross separately and the individual tests of significance are presented in Table 3. Although these tests of significance

TABLE 3

Heterosis as exhibited by each cross separately

Cross	28-day weight			70-day weight		
	Mean for linebreds	Mean for crosses	Difference	Mean for linebreds	Mean for crosses	Difference
AB	61.1	61.6	.5	194.8	203.9	9.1
AD	54.8	63.3	8.5**	173.0	186.0	13.0
AH	58.4	63.7	5.3*	173.8	188.2	14.4*
BD	60.0	62.1	2.1	192.0	198.6	6.6
BH	63.6	59.1	—4.5	192.8	194.4	1.6
DH	57.3	58.6	1.3	171.0	174.3	3.3
Over-all	59.2	61.4	2.2	182.9	190.9	8.0*

* Significant at .05 level of probability.
** Significant at .01 level of probability.

are not independent, since the linebreds are each used three times, they are of considerable interest because of the variability in heterosis from cross to cross. The average heterosis for crosses involving line A was 4.8 gms for 28-day weight and 12.2 gms for 70-day weight; corresponding values for the B line were —0.6 gms and 5.8 gms; line D, 4.0 gms and 7.6 gms; and line H, 0.7 gms and 6.4 gms. Hence, line A performed the best in crosses and line B, which was the best inbred, performed the poorest in crosses.

The highly significant differences among linebreds for 28- and 70-day weight was consistent with previous experience with these lines. Line H was 5.0 gms

heavier than line A at 28 days and 4.1 gms lighter at 70 days. This may have reflected a difference in maternal ability. Although only linecross litters were used to measure maternal ability, all dams were inbred, i.e., no linecross females produced litters. Linebred progeny in line H may have received better maternal effects prior to weaning and a compensating effect occurred after weaning. YAO and EATON (1954) attributed a similar observation in inbred strains of rabbits to different postnatal growth rates.

General combining ability effects are highly significant at 28 days, but not significant at 70 days. Preweaning differences in maternal ability and a postweaning compensating effect might also account for these results. HENDERSON (1949) studied single crosses among 12 inbred lines of swine and found that general combining ability accounted for not more than five percent of the variation among line crosses with respect to litter size and weight at birth, 21, 56, and 154 days.

There was no evidence of either specific combining ability or sex-linked effects at either age. HENDERSON (1949) found specific effects (dominance and epistasis) to account for five to 15 percent of the variation among swine crosses. EATON, NEVILLE, and DICKERSON (1950) made the 72 possible crosses among nine inbred lines of mice. They found that specific linecross combination effects were important though not significant for viability and total litter weight, but not for individual mouse weights. It is quite possible that crosses among more rat lines might reveal some specific effects. HENDERSON (1949) found no evidence of sex-linked effects.

Maternal effects were highly significant at 28 and 70 days. BAILEY (1954) using a cross-nursing technique also found maternal effects to be important at these ages. Important maternal effectes have been found in rabbits by VENGE (1950) and by YAO and EATON (1954), and in mice by MARSHAK (1936). HENDERSON (1949), however, found no evidence of maternal effects in single crosses among 12 inbred lines of swine. This latter finding is not inconsistent with the observation of CHAMBERS and WHATLEY (1951) of heterosis in productivity of linecross female swine.

The relation between the least squares constants for maternal effects and general combining ability effects for 28- and 70-day weights is shown in Table 4. These constants are expected to be negatively correlated ($r = -0.57$) because maternal effects and general combining ability effects are highly confounded and the correlation is obtained from the elements of the inverse matrix. The difference between the observed and the expected r may provide some information concerning the genetic correlation between maternal and general combining ability effects. With only four lines the information is indeed meager, but it is of interest to note the difference in the estimate of the genetic correlation from 28- to 70-day weight.

If all crossbred offspring exhibit the same amount of heterosis, the differences among the maternal effects as measured in crossbreds will be the same as expected in linebreds. Also, if the model has properly described the effects in crosses, the

TABLE 4

Correlation between maternal and general combining ability effects

Line	28-day weight			70-day weight		
	Maternal		GCA	Maternal		GCA
A	—1.7	. .	3.0	—9.8	. .	7.6
B	1.5	. .	—1.4	20.8	. .	1.7
D	—5.8	. .	2.8	—8.8	. .	—2.5
H	—6.0	. .	—4.4	—2.2	.	—6.8
Observed r		—.93			—.01	
Expected r		—.57			—.57	
Difference		—.36			+.56	

additively genetic effects as estimated from crosses will provide an unbiased estimate of the transmittible genic effects for linebreds as well as crosses. Hence, the differences between the $\hat{p}_{1k}$ and the $2\hat{g}_{2k} + \hat{m}_{2l}$ will indicate whether non-additive effects are operative within line and/or whether the lines tend to rank differently with respect to maternal effects when linebred or crossbred progeny are being reared. Table 5 compares the $2\hat{g}_{2k} + \hat{m}_{2l}$ with the $\hat{p}_{1k}$ for all four lines and both traits. The difference between the observed and expected linebred constant is highly significant for lines A and B at 28 days. The correlation between observed and expected constants is — 0.51 at 28 days and + 0.91 at 70 days.

TABLE 5

Comparison of the $2\hat{g}_{2k}+\hat{m}_{2l}$ *with the* $\hat{p}_{lk}$

Line	28-day weight			70-day weight		
	$2\hat{g}_{2k}+\hat{m}_{2l}$	$\hat{p}_{1k}$	Difference	$2\hat{g}_{2k}+\hat{m}_{2l}$	$\hat{p}_{1k}$	Difference
A	4.4	—3.3	—7.7**	5.4	— 7.1	—12.5
B	—1.3	7.1	8.4**	24.1	30.9	6.8
D	— .3	—5.5	—5.2	—13.8	—12.6	1.2
H	—2.8	1.7	4.5	—15.7	—11.2	4.5

** Significant at the .01 level of probability.

These results clearly suggest that, for 28-day weight, these lines do rank differently with respect to maternal effects when the dams are rearing crossbred progeny than when they are rearing linebred progeny. In other words, an interaction of maternal effects with mating system is indicated.

Except for the lack of specific effects in these data and their presence in swine, and the presence of maternal effects in these data and their absence in swine, these results are in general agreement with those found in other mammalian species. The data are clearly insufficient to warrant any broad generalizations with respect to hybrid vigor in crosses among inbred lines of rats.

SUMMARY AND CONCLUSIONS

The 16 possible combinations including inbreds and reciprocal crosses were made among four inbred lines of rats. The analytical method provided estimates of the effects of sex, heterosis, lines, general and specific combining ability, sex linkage, and maternal effects on 28- and 70-day body weight. Effects of sex, lines and maternal ability were highly significant at 28 and 70 days. Heterosis was significant at 70 but not at 28 days. General combining ability was highly significant at 28 but not at all significant at 70 days. There was no evidence of specific combining ability or sex linkage effects. An interaction of maternal effects and mating system with respect to 28-day weight was indicated.

LITERATURE CITED

BAILEY, D. W., 1954 The inheritance of maternal influences on growth of the rat. Ph.D. Thesis. University of California Library.

BECKER, W. A., 1957 The genetics of body weight in rats: I. Selection progress and linkage. II. Sources of phenotypic variance. Ph.D. Thesis. University of California Library.

CHAMBERS, D., and J. A. WHATLEY, JR., 1951 Heterosis in crosses of inbred lines of Duroc swine. J. Animal Sci. **10**: 505–515.

DICKERSON, G. E., J. L. LUSH, and C. C. CULBERTSON, 1946 Hybrid vigor in single crosses between inbred lines of Poland China swine. J. Animal Sci. **5**: 16–24.

DICKERSON, G. E., 1952 Inbred lines for heterosis tests? Ch. 21. *Heterosis*. Edited by JOHN GOWEN. Iowa State College Press. Ames, Iowa.

DUNCAN, D. B., 1955 Multiple range and multiple F tests. Biometrics **11**: 1–42.

EATON, N. E., W. E. NEVILLE, and G. E. DICKERSON, 1950 General and specific combining abilities in mouse crosses. J. Animal Sci. (Abst.) **9**: 636–637.

HENDERSON, C. R., 1948 Estimation of general, specific and maternal combining abilities in crosses among inbred lines of swine. Unpublished Ph.D. Thesis. Iowa State College Library. Ames.

1949 Estimation of general, specific and maternal combining abilities in crosses among inbred lines of swine. J. Animal Sci. (Abst.) **8**: 606.

1952 Specific and general combining ability. Ch. 22. *Heterosis*. Edited by JOHN GOWEN. Iowa State College Press. Ames, Iowa.

KING, H. D., 1915 The growth and variability in body weight of the Albino rat. Anat. Record **9**: 751–776.

KRAMER, C. Y., 1957 Extension of multiple range tests to group correlated adjusted means. Biometrics **13**: 13–18.

LIVESAY, E. A., 1930 An experimental study of hybrid vigor on heterosis in rats. Genetics **15**: 17–54.

MARSHAK, A. G., 1936 Growth differences in reciprocal hybrids and cytoplasmic influence on growth in mice. J. Exptl. Zool. **72**: 497–510.

VENGE, O., 1950 Studies of the maternal influence on the birth weight in rabbits. Acta Zool. **31**: 1–148.

YAO, T. S., and O. N. EATON, 1954 Heterosis in the birth weight and slaughter weight in rabbits. Genetics **39**: 667–676.