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GENETIC EFFECTS OF AGE ON PERFORMANCE IN RANDOM-BRED MICE

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Methods of selection and evaluation of response are a major topic in animal breeding. Classical examples are the estimation of breeding values from progeny records (Lush, 1945) and repeat mating designs for measuring response to selection (Goodwin, Dickerson and Lamoreux, 1960). The reliability of these methods depends on animals and their gametes being genetically constant during their reproductive period.

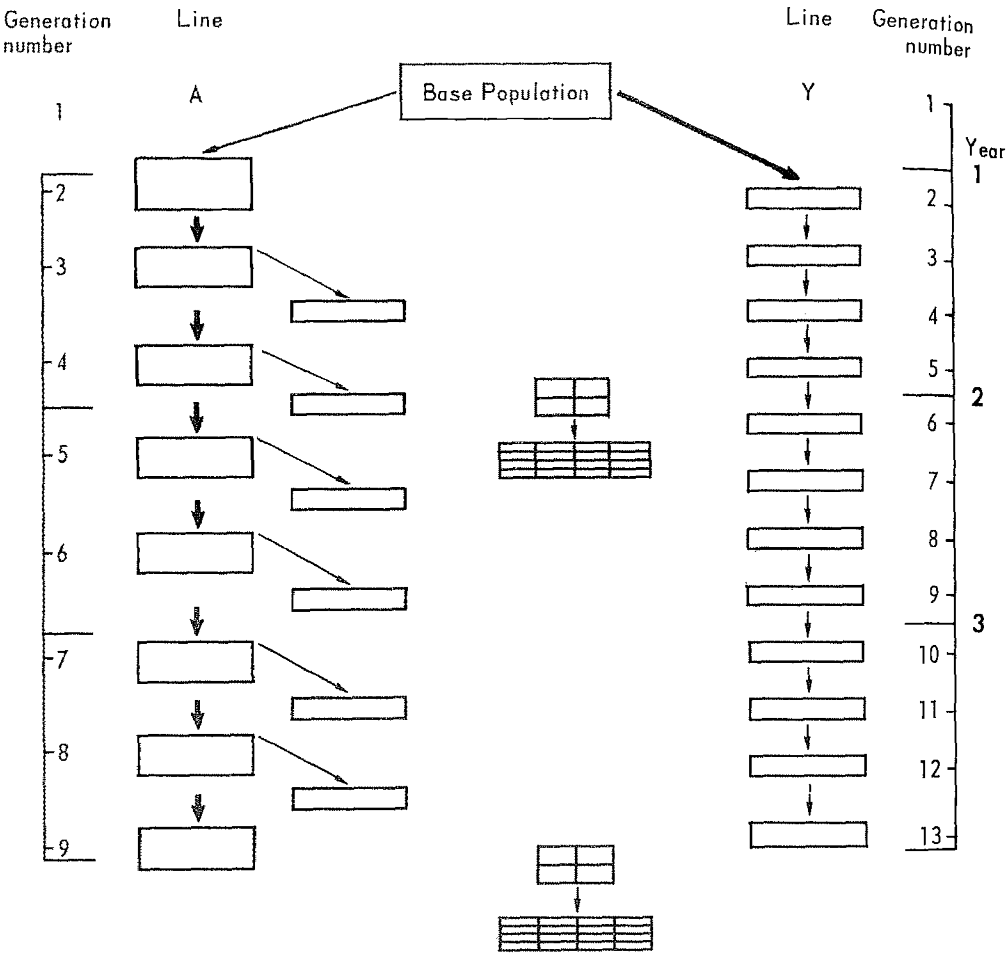
There is evidence that the offspring from young parents are on the average, genetically different from those born to the same but aged parents. In man, the incidence of Down's syndrome increases with maternal age and some cases are due to trisomy (Ford, 1962). In Drosophila, segregation ratio changed with paternal age when males were heterozygous for the Segregation-Distorter (Sandler and Hiraizumi, 1961). In mice, two inbred lines differing in age at mating produced progeny that differed in the appearance of fibrosarcomas (Strong, 1954). Other relevant reports have been reviewed by Parsons (1964).

The main objective of the present study was to explore genetic changes in growth caused by successive generations of aged parents.

MATERIALS AND METHODS

The base population was originally synthesized from all possible crosses of four inbred strains and subsequently maintained by random mating for 12 generations. From this randombred population, one female and one male were chosen randomly from each of 78 litters. These mice (Generation 1) were used for random pair matings to produce two litters of first and second parity. The first and second litters formed the A and Y lines, respectively (Figure 1). Mice from first litter were mated at 20 weeks of age and mice from second litters were mated at ten weeks of age. In each case, one male from each pair of litters and one female from each litter were selected at random. The foundation of the two lines (A2 and Y2) contained 78 females and 39 males as breeders. They were randomly mated within line to produce litters contemporaneously (A3 and Y3). Selecting one female from each dam and one male from each sire family, random matings were performed

Fig.1. Diagram illustrating the maintenance of the A and Y lines



within A3 and Y3, and in subsequent generations. After Generation 3, the first litters A line were discarded at weaning and the second litters provided breeding stock for the subsequent generation, while the Y line was maintained by breeding from first litters. The two ages of mating were ten and 20 weeks for the first and second litters, respectively. Throughout this experiment, two lines were maintained with as similar ancestry as possible: pedigreed breeders in the Y line were randomly mated, and those in the A line at the same generation were mated in a similar manner. Matings were mostly in the ratio of one male to two females, and males were cohabited with females for 16 days. At birth, litter size was standardized to eight. When litter size was less than eight, the young from another litter were fostered. The fostered young were excluded from the analysis and matings.

The A4 and Y5 were used for a 2 x 2 diallel mating to produce their first litters. The resulting offspring classified as AA, AY, YA and YY were contemporary and at ten weeks of age they were used for a 4 x 4 diallel mating to produce first litters. Similar matings were conducted starting with A9 and Y13.

Measurements : Body weights of individual young at birth and at 12, 20 and 42 days of age were recorded. Since body weight after 20 days was significantly different between sexes, individual body weight of the young at 42 days was converted to the "neutral" value using the formulae of Falconer and King (1953) as follows.

Female : Individual weight x $(1 + r)/2$
Male : Individual weight x $[1 + (1/r)]/2$

where r stands for the ratio of the average male weight to the average female weight within generation. The mean and variance on logarithmic scale were calculated using the formulae of Wright (1952) as follows :

$$\text{Mean}: \overline{\log x} = \log_{10} \bar{x} - \log_{10} (1+C^2)/2$$

$$\text{Variance}: s^2_{\log_{10} x} = .43431 \log_{10} (1+C^2),$$

where $\bar{x}$ and C stand for the mean and coefficient of variation respectively, on actual gram scale.

Expected differences between the two lines: The main premises in the present study are, (1) age and/or parity affect genotypes and/or cytoplasmic factors, (2) genetic changes due to age and/or parity are accumulated over generations, and (3) genetic differences between lines due to segregation are little. From these, one can expect that (a) the contemporary A3 and Y3 and their first litters differ by the genetic effects of parity from one generation and (b) A9 and Y13 differ by any accumulated genetic effects from six generations differing in age and five generations differing in parity. The genetic effects of age and/or parity were defined as any transmittable changes occurring in aged parents. Estimating such effects was attempted by comparing the offspring produced by AA, AY, YA and YY parents of the same age. All possible matings of the four types (AA, AY, YA and YY) at ten weeks of age produced six genotypes of offspring. They were P_A (from AA x AA), P_Y (YY x YY) F_1 (YY x AA and AA x YY), F_2 (YA x YA, YA x AY, AY x YA and AY x AY), B_Y (YA x YY, AY x YY, YY x YA and YY x AY) and B_A (YA x AA, AY x AA, AA x YA and AA x AY).

RESULTS AND DISCUSSIONS

Difference between the A and Y lines at Generations 3 and 4: Body weight of dams, litter size and body weight of young are shown in Table 1. Young mothers (Y2) had a higher conception rate and produced

Table 1. Means and standard errors in the A and Y lines

Trait	Generation for dams	Line A	Line Y
Body weight of dams (g)	2	32.0 ± .41 (73/78) π	30.7 ± .34 (77/78)
Litter size at birth		8.7 ± .29	9.3 ± .23
Body weight of young (g)			
Birth (0 day)		1.49 ± .01	1.50 ± .01
12 days		7.9 ± .04	8.0 ± .03
20 days		11.7 ± .05	11.8 ± .05
Body weight of dams (g)	3	30.9 ± .39 (68/72)	30.9 ± .53 (71/72)
Litter size at birth		8.4 ± .34	8.9 ± .23
Body weight of young (g)			
Birth (0 day)		1.51 ± .01	1.56 ± .01
12 days		7.8 ± .05**	8.3 ± .05
20 days		11.9 ± .06*	12.3 ± .07

π Number of dams that produced litters/number of dams used for mating

**, *Significantly different between the A and Y lines at the 1 and 5% level, respectively.

Table 2. Mean body weights of offspring from AA, AY, YA and YY produced by A4 and Y5

Age type		Body weight of dam (g)	Litter size at birth	Number of litters	Body weight of young (g) at Birth (0 day)	12 days	20 days	42 days
Sire	AA	29.6 ± .30**	8.4 ± .20	54	1.55 ± .01	7.6 ± .01	11.0 ± .05	23.3 ± .08
	AY*	29.1 .31	8.9 .20	59	1.50 .01	7.6 .04	11.0 .05	22.9 .09
	YA	29.3 .31	8.2 .21	61	1.54 .01	7.8 .04	11.1 .05	23.3 .09
	YY	30.0 .32	8.7 .22	64	1.53 .01	7.7 .04	11.0 .05	23.3 .08
Dam	AA	29.4 ± .31	8.6 ± .20	64	1.52 ± .01	7.7 ± .04	11.0 ± .05	23.3 ± .09
	AY	29.6 .31	8.3 .21	55	1.57 .01	7.8 .04	11.2 .05	23.4 .09
	YA	29.3 .32	8.9 .21	58	1.50 .01	7.5 .04	10.8 .05	22.9 .08
	YY	29.8 .30	8.4 .20	61	1.53 .01	7.7 .04	11.1 .05	23.2 .08

* Sires produced by males of the A line and females of the Y line

** Mean ± standard error

larger litters than aged mothers (A2). Neither conception rate nor litter size was significantly different between Y2 and A2 ($P > 0.05$). Apparently, aging without parity has an adverse effect on reproduction which could be due to excessive fatness. Although reproduction differed between the A2 and Y2 dams, body weights of the young (A3 and Y3) were similar at birth and at 12 and 20 days. However, A4 and Y4 (first litters from A3 and Y3 dams) differed significantly in body weights at 12 days ($P < 0.01$) and 20 days ($P < 0.05$). Covariance analysis revealed that the difference in birth weight did not account for the differences in body weights at 12 and 20 days. Since 12-day weight can be employed as a measure of postnatal maternal effects (Young, Legates and Farthing, 1965), the finding suggests that females born to young virgins (Y2) were higher in milk production than those born to aged virgins (A2).

Performance of offspring from AA, AY, YA and YY produced by A4 and Y5 : The mean body weight of the offspring classified by parental types (AA, AY, YA and YY) is shown in Table 2. Body weight of the young was similar among four types of sire or dam at each of the four ages. Analysis of variance (Table 3) using a randomized complete block model (fixed model) revealed that the mean weights did not differ significantly among

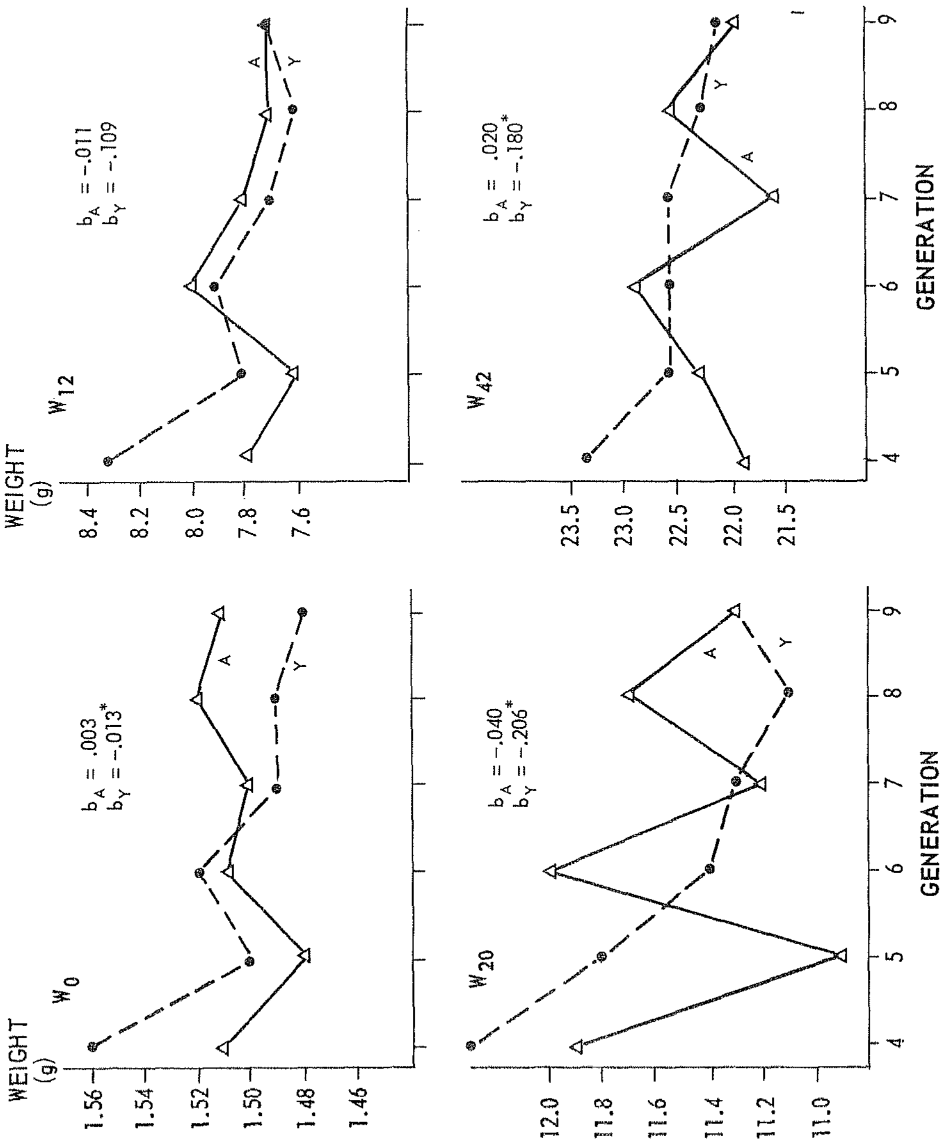
Table 3. Analysis of variance for body weight of young from mice (AA, AY, YA and YY) produced by A4 and Y5

Age type of parents involved	Source of variation	df	Mean squares Birth weight	12-day weight	20-day weight	42-day weight
AA, AY	Replicate	17	0.25	4.43	13.02	49.39**
YA and	Age type of sire (S)	3	0.14	4.59	2.21	18.57
YY	Age type of dam (D)	3	0.35	7.48	10.47	19.56
	S x D	9	0.17	1.71	5.29	14.46
	Error	238	0.14**	3.74**	7.51**	16.47**
	Residual	1,492	0.01	0.28	0.64	8.58
AA	Replicate	17	0.13	9.71**	17.27*	45.84**
	Age type of sire (S)	1	0.03	2.62	1.93	26.27
and	Age type of dam (D)	1	0.02	1.42	5.88	0.12
	S x D	1	0.09	1.25	1.58	0.64
YY	Error	42	0.10**	4.37**	9.46**	21.07**
	Residual	386	0.01	0.31	0.64	8.16

*, ** Significant $P < 0.05$ and < 0.01 respectively.

four types (AA, AY, YA and YY), nor among two types (AA and YY). It was concluded from these findings that the observed differences between the offspring from A3 and Y3 in 12-day and 20-day weight (Table 1)

Fig. 2. Changes in mean body weight with generations



were due to genetic differences that existed temporarily, or sampling errors.

Changes in the mean body weight over generations : During the first nine generations, numbers of dams and sires decreased by infertility or mortality from 78 to 58 and 39 to 29, respectively, in both the A and Y lines. With breeding procedures used, the degree of inbreeding was expected to be similar in the A and Y lines. If natural selection existed it was assumed to have affected both lines similarly. Changes in the average weights with generations for first litters of the A and Y lines are shown in Figure 2. Significant regressions ($P < 0.05$) of the average weight on generation number existed for the Y line but not for the A line. However, the A line was considerably lower in weights at the four ages than the Y line at Generation 4, and by excluding this generation there was no significant difference between the regressions for the two lines.

Performance of the offspring from AA, AY, YA and YY produced by A9 and Y13 : Body weight of young is influenced by post natal maternal environments which are controlled by the mother's genotype (Young, Legates and Farthing, 1965). Whether the difference in mother's type (AA, AY, YA and YY) is important in body weight of young was examined. On an additive-dominance model (Mather and Jinks, 1971) the expected means of offspring can be expressed (Table 4) in terms of the average

Table 4. The expected means on an additive-dominance model in the presence of maternal effects

Parents		Offspring	Expected genetic contribution to the mean of offspring	
Sire x dam	Genotype	genotype	Offspring	Mother
YY x YY	$P_Y \times P_Y$	P_Y	m + [d]	+[dm]
AA x AA	$P_A \times P_A$	P_A	m - [d]	-[dm]
YY x AA	$P_Y \times P_A$	F_1	m + [h]	-[dm]
AA x YY	$P_A \times P_Y$	F_1	m + [h]	+[dm]
YA x YA YA x AY AY x YA AY x AY	$F_1 \times F_1$	F_2	m + [h]/2	+[hm]
YA x YY AY x YY	$F_1 \times P_Y$	B_Y	m + [d]/2 + [h]/2	+[dm]
YY x YA	$P_Y \times F_1$	B_Y	m + [d]/2 + [h]/2	+[hm]
YA x AA AY x AA	$F_1 \times P_A$	B_A	m - [d]/2 + [h]/2	-[dm]
AA x YA AA x AY	$P_A \times F_1$	B_A	m - [d]/2 + [h]/2	-[hm]

For symbols see text.

Table 5. Means ($\bar{x}$) and within-litter variances (s_w^2) of body weight for F_1 and backcrosses (B_Y and B_A)

Parents Sire x Dam	Geno-type	No.*	Scale**	Offspring Birth weight $\bar{x}$	Birth weight s_w^2	12-day weight $\bar{x}$	12-day weight s_w^2	20-day weight $\bar{x}$	20-day weight s_w^2	42-day weight $\bar{x}$	42-day weight s_w^2
YY x AA	F_1	32	Actual	1.44	.008	8.10	.14	12.20	.56	21.47	2.98
			Log	.1575	.00074	.9080	.00040	1.0856	.00070	1.3305	.00120
AA x YY		48	Actual	1.49	.008	8.16	.24	11.60	.37	22.74	2.57
			Log	.1724	.00066	.9109	.00068	1.0639	.00053	1.3557	.00092
F_1 x YY (AY or YA)	B_Y	85	Actual	1.47	.011	8.42	.23	12.21	.54	22.40	3.75
			Log	.1661	.00098	.9243	.00059	1.0860	.00065	1.3487	.00141
YY x F_1 (AY or YA)		84	Actual	1.49	.012	8.35	.26	11.92	.56	22.58	2.59
			Log	.1721	.00096	.9205	.00071	1.0745	.00076	1.3527	.00072
F_1 x AA (AY or YA)	B_A	93	Actual	1.52	.011	8.11	.29	11.50	.37	22.07	2.14
			Log	.1794	.00086	.9082	.00049	1.5080	.00052	1.3428	.00084
AA x F_1 (AY or YA)		87	Actual	1.51	.011	7.69	.19	11.03	.46	21.88	2.44
			Log	.1765	.00093	.8878	.00061	1.0416	.00072	1.3389	.00097

* Number of mice at birth ** in g.

Table 6. Means ($\bar{x}$) and within-litter variances (s_w^2) of body weight for each offspring genotype

Genotype	No.	Birth weight $\bar{x}$ obs.	$\bar{x}$ theor.	s_w^2	$\overline{\log x}$ obs.	$\overline{\log x}$ theor.	$s^2_{\log x}$	12-day weight $\bar{x}$ obs.	$\bar{x}$ theor.	s_w^2	$\overline{\log x}$ obs.	$\overline{\log x}$ theor.	$s^2_{\log x}$
P_Y	46	1.55		.006	.1898		.00043	8.31		.40	.9183		.00109
P_A	46	1.52		.019	.1801		.00156	8.37		.20	.9221		.00053
P_M	92	1.54		.013	.1850		.00100	8.34		.30	.9202		.00081
F_1	80	1.47		.008	.1650		.00070	8.13		.19	.9095		.00054
F_2	184	1.46	1.51	.014	.1615	.1750	.00118	8.06	8.24	.22	.9055	.9149	.00064
B_Y	169	1.48	1.51	.011	.1691	.1774	.00097	8.38	8.22	.24	.9224	.9139	.00065
B_A	180	1.51	1.50	.011	.1779	.1726	.00089	7.92	8.25	.24	.8980	.9158	.00055
		20-day weight						42-day weight					
P_Y	45	12.01		.83	1.0783		.00109	21.98		4.52	1.3400		.00177
P_A	46	11.22		.38	1.0493		.00057	19.96		1.91	1.2991		.00090
P_M	91	11.62		.61	1.0638		.00083	20.97		3.22	1.3196		.00134
F_1	80	11.90		.47	1.0748		.00062	22.11		2.78	1.3431		.00106
F_2	183	11.47	11.76	.54	1.0584	1.0693	.00078	21.97	21.54	3.50	1.3398	1.3314	.00136
B_Y	165	12.06	11.96	.55	1.0802	1.0766	.00070	22.40	22.05	3.17	1.3507	1.3416	.00106
B_A	178	11.24	11.56	.41	1.0498	1.0621	.00062	21.98	21.04	2.29	1.3408	1.3211	.00090

value (m) of the two lines (Y and A), additive gene effects (d), dominance effects (h), maternal additive gene effects (dm), and maternal dominance effects (hm). The F_1 hybrids from the reciprocal matings (YY x AA and AA x YY) differ by twice the maternal additive gene effects (2 dm). Therefore, comparison of the means for two F_1s examines whether the difference in maternal additive gene effects is important in body weight of young. Likewise, comparison of the means for two backcrosses (P_Y x F_1 vs. F_1 x P_Y) and the comparison of the means for two other backcrosses (P_A x F_1 vs. F_1 x P_A) examine whether the difference in maternal additive and maternal dominance effects is important in body weight of young. The means and within-litter variances on actual and logarithmic scales were calculated separately for F_1 and backcrosses (Table 5). The within-litter variance measures variation under uniform environments.

A conventional t-test revealed that the difference in body weight between the two F_1-s, (P_Y x P_A) and (P_A x P_Y), was significant only at 20 day ($P < 0.05$) on both actual and logarithmic scales. The backcrosses from P_Y x F_1 and F_1 x P_Y did not differ in any traits examined on both scales. The backcrosses from P_A x F_1 and F_1 x P_A ($P < 0.01$) differed only in 12-day and 20-day weight. It was concluded that the difference in mother's age type (AA, AY, YA and YY) was not important in body weight of offspring from mothers mated at the same age.

The means and within-litter variances for the six genotypes of offspring (P_Y, P_A, F_1, F_2, B_Y and B_A) are shown in Table 6, where P denotes mid-parent (the averaged value of P_Y and P_A), and theoretical means for F_2 and backcrosses (B_Y and B_A) were calculated from the formulae of Wright (1952). In comparision of the observed mean with the theoretically expected, the mean on logarithmic scale was in slightly better agreement with the theoretically expected than that on actual scale except for birth weight. However, tests of scales using the methods of Mather and Jinks (1971) revealed that the value of actual and logarithmic scales was virtually the same except in one test for 12-day and 42-day weight. Both actual and logarithmic scales were used in the present study. There was a considerable difference in variance among the six genotypes (P_Y, P_A, F_1, F_2, B_Y and B_A). Bartlett's tests revealed that variance differed significantly ($P < 0.05$) among genotypes, except for 12-day weight on actual scale and 20-day weight on logarithmic scale.

Regression of the mean body weight on coded genetic effects of age : The mating of the four types (AA, AY, YA and YY) in all possible combinations produced young that were different in the accumulated genetic effects of age. These effects were coded for analysis purposes, as illustrated in Table 7. For example, the offspring produced by YY x YY (P_Y) and AA x AA (P_A) were coded as zero and two because they had zero "A" and two "A", respectively. The F_1, F_2, B_Y and B_A offspring were coded as 1, 1, 0.5 and 1.5 respectively. If the regression of the mean weights of offspring on their coded "A" effects is significant, the weights are genetically affected by accumulated parent age effects.

Fig. 3. Relationship between body weight and genetic effects of age

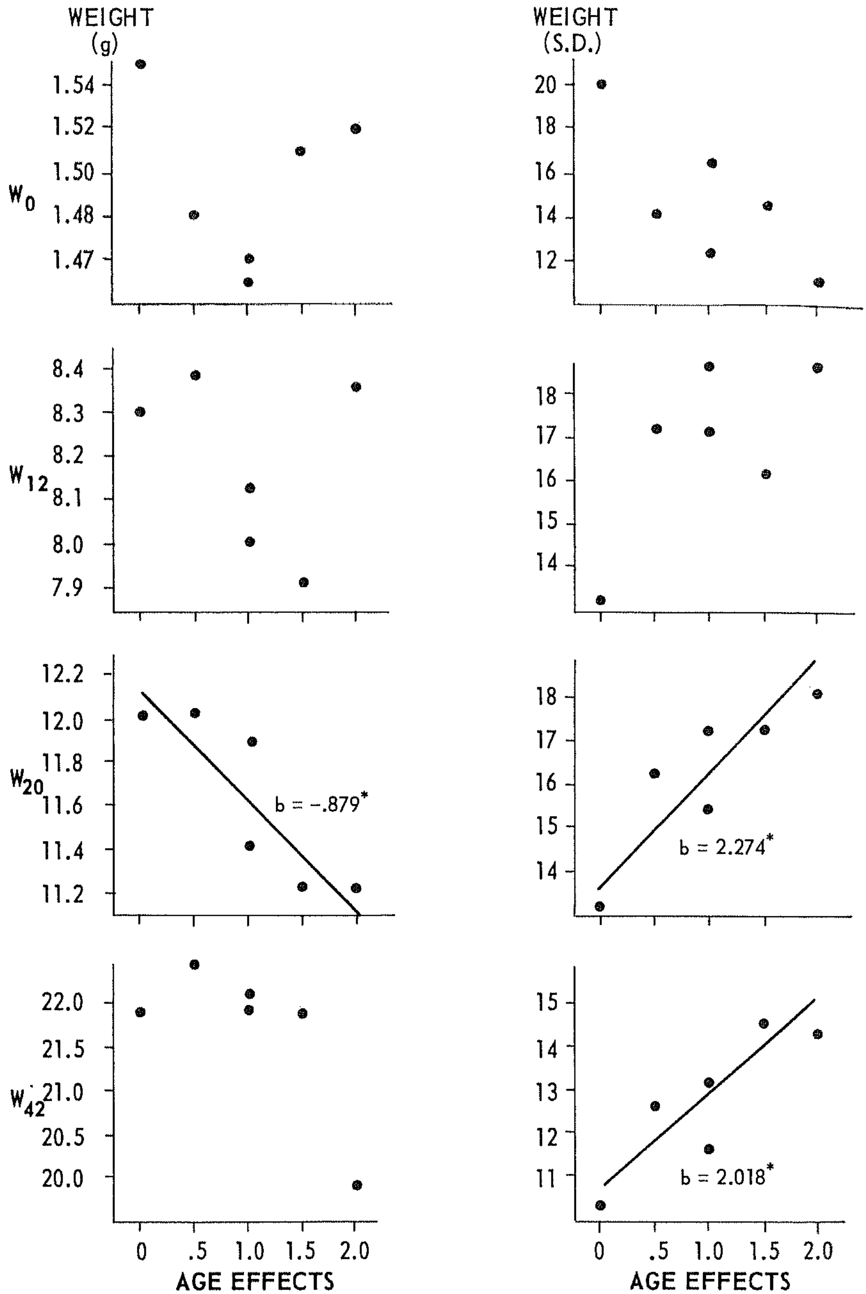


Table 7. The coded genetic effects of age for offspring produced by various types of parents

Parents Sire x Dam	Genotype	Offspring Type	Coded "A" effects
YY x YY	P_Y	YY	0
AA x AA	P_A	AA	2
YY x AA AA x YY	F_1	AY	1
YY x YA YA x AY AY x YA AY x AY	F_2	.25YY .25AA .50AY	1
YA x YY AY x YY YY x YA YY x AY	B_Y	.50YY .50AY	.5
YA x AA AY x AA AA x YA AA x AY	B_A	.50AY .50AA	1.5

The regression was calculated in two ways. One was to relate the actual means to their coded "A" effects, and the other was to relate the actual means divided by their respective standard deviations to coded "A" effects. Results are shown in Figure 3. The regression of actual birth weight and 12-day weight on coded "A" effects indicated no definite trends. However, the regression was significant ($P < 0.05$) for standard deviates of 20-day and 42-day weight, and actual 20-day weight. For 20-day and 42-day weight the two types (AA and YY) differed by approximately four standard deviates in favour of the AA type. On logarithmic scale a similar regression was observed, but the significance ($P < 0.05$) was confirmed only for the 20-day log weight.

It was concluded from all analyses that birth weight and 12-day weight were not genetically affected by accumulated parent age effects. Results from the analysis of coded genetic effects on 20- and 42-day weights (Figure 3) were inconclusive. These traits have greater genetic variation than birth weight and 12-day weight (Young, Legates and Farthing, 1965; Young and Legates, 1965). Therefore, it is likely that random drift effects were greater in 20-day and 42-day weights, and made the results inconclusive in the present study. Taking into account the drift effect and the fact that regression of the mean body weights on generations did not differ between the A and Y lines, we conclude that genetic age effects do not appear to exist for 20-day and 42-day weights.

Random-bred mice can be used when effects of age on various genotypes are to be examined. However, it causes a difficulty in detecting real age effects since differences between contemporary lines contain genetic age effects and/or random drift effects. Experiments using replicated lines would provide an estimate of genetic age effects that are separated from random drift effects, but they require consideration to facilities, particularly when lines are to be maintained for many generations. In the present study, attempts were made to minimize genetic differences between lines due to random drift under the conditions available : sampling of one female and one male offspring within dam and sire family respectively, and maintaining of the A and Y lines with as similar an ancestry as possible. However, random drift effects that are more prominent for 20-day and 42-day weights made the retults inconclusive for these traits. Use of genetically uniform mice (inbred or F_1 hybrid) for studying age effects has advantages in that random-drift effects are unimportant, and mice can be mated at older ages (say 350 days) to produce extreme genetic effects of age, if any. Such studies are underway.

SUMMARY

Two lines of mice (A and Y) differing in age at mating were maintained with as similar ancestry as possible for nine and 13 generations, and were used for diallel matings to contemporaneously produce various genotypes of offspring (A, Y, F_1, F_2, and backcross). Body weights of offspring at birth, and at 12, 20 and 42 days of age were recorded, and data on actual and logarithmic scales were analysed Regression of the mean body weight for the respective genotype on the coded A-line gene effects was significant ($P < 0.05$) only in 20-day weight on both actual and logarithmic scales. When the mean body weight was expressed in standard deviation unit, regression was significant ($P < 0.05$) for 20-day and 42-day weight on actual scale. It was concluded that birth weight and 12-day weight were not genetically affected by accumulated parent age effects. The results inconclusive with respect to 20-day and 42-day weight were discussed.

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