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Genetic aspects of fertility and endocrine organ size in rats*

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SUMMARY

An investigation of the genetic aspects of fertility was conducted among inbred and hybrid generations of rats. The high-fertility LEW strain and the low fertility CAS strain were crossed and their hybrids inbred for four generations. Litter size, ovulation rate, sterility, and the weights of the thyroid, pituitary, adrenals, testes, seminal vesicles, ventral prostate, uterus, and ovaries were analysed in inbred and hybrid rats for evidence of strain differences and heterosis and in successive generations of sib matings for inbreeding depression.

CAS females produced smaller litters and had smaller thyroids, pituitaries, adrenals, ovaries, and uteri than LEW females. CAS males had larger testes but smaller adrenals than LEW males. Results of crosses included heterosis for female pituitary and ovary weights, but inbreeding depression for the weights of male adrenals, seminal vesicles, and ventral prostates, and female thyroids and uteri. Ovulation rate did not differ between strains and was not an important determinant of litter size in this study.

The decrease in litter size as a result of inbreeding was due partly to the inbreeding of the parents and partly to the inbreeding of the litter.

1. INTRODUCTION

Most of the work on the genetic aspects of reproductive performance in mammals has concentrated on ovulation rate, litter size, and post-ovulation events such as implantation and mortality of embryos. Jinks & Broadhurst (1963) found that litter size in rats is predominantly maternal and not affected by the sire or genotype of the litter. In addition to a significant difference in litter size, Cole & Casady (1947) noted differences in the weights of the ovaries, thyroids, and adrenals of two strains of rats of common ancestry.

In mice, the decrease in litter size due to inbreeding has been reported by Roberts (1960) to be 0·49 pups and by Bowman & Falconer (1960) to be 0·56 pups per 10% increase in the inbreeding coefficient. Falconer & Roberts (1960) found that ovulation rate in mice was not influenced by inbreeding whereas McCarthy (1967) found that it was.

Craig, Casida & Chapman (1954) reported low libido as a cause of male infertility

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in a strain of rats. In addition, they found that inbred non-breeders had heavier testes but lighter seminal vesicles than inbred breeders. Similarly, Sreebny *et al.* (1959) found the testes of CAS rats, which were used in this present study, to be heavier than in the CAR strain, which was superior to the CAS strain in its reproductive performance.

This present work is an extension of Hoornbeek's (1968) study of mating success and litter size variation. It was designed to study the genetics of various components of reproductive performance, namely litter size, ovulation rate, and sterility, while simultaneously studying the weights of organs of the reproductive and endocrine systems. The organs studied were thyroids, pituitaries, adrenals, ovaries, uteri, testes, seminal vesicles, and ventral prostates. The characters were studied in two inbred strains to determine if there were strain differences. To determine if there was any evidence of directional dominance of the genes affecting each character the two strains were crossed and the characters studied in F_1–F_4 generations. The complementary, or opposite, phenomena of heterosis and inbreeding depression are manifestations of directional dominance (Falconer, 1960).

2. MATERIALS AND METHODS

Two inbred strains of rats (*Rattus norvegicus*) were used as the parental generations in this study. The inbred LEW strain is noted for its good reproductive ability, whereas the CAS strain is noted for its susceptibility to dental caries and poor reproductive ability (Robinson, 1965; Hoornbeek, 1968). The two strains were crossed and their hybrids inbred for four generations without selection.

As no fertile CAS males were available at the start of this experiment, all F_1 hybrids were from CAS dams with LEW sires. Other than the CAS × LEW cross, all matings were full brother–sister. The calculated inbreeding coefficients of successive generations F_1 through F_5 are 0, 0·5, 0·5, 0·63, and 0·69, respectively. The F_2 and F_3 generations have the same inbreeding coefficient, and therefore in most instances the F_2 and F_3 data were pooled and designated $F_{2,3}$.

The rats were housed in wire cages on wood shavings, fed Purina Lab Chow, and given a constant supply of water. The young were weaned and weighed at 25 days of age and separated by sex until 100 days, at which time they were weighed again and either mated or held for autopsy. The average age at autopsy was 115 days.

Females were autopsied in metestrus as indicated by vaginal smears. Corpora haemorrhagica, mature follicles, and/or young corpora lutea were all considered when determining ovulation rate. Body weight and the weights of the thyroid, pituitary, adrenals, ovaries, and uterus (drained) were also recorded.

For males, body weight and the weights of the thyroid, pituitary, adrenals, testes, seminal vesicles (drained) and ventral prostate (drained) were recorded. Smears, in normal saline, of the fluid from the cauda epididymis showed that all males autopsied had normal, motile sperm.

Body weight, litter size, ovulation rate, sterility, and the weights of the endo-

crine and accessory organs were analysed statistically to determine differences between inbred and hybrid generations. For paired organs such as adrenals, gonads, and seminal vesicles the weights were expressed as the sum of the two organs. Ovulation rate was also expressed as the sum of the two ovaries. Litter size was taken as the total number born in a dam's first litter.

When females failed to become pregnant after being paired with a male for at least a 21-day trial period, both the male and female were paired with known fertile mates for an additional trial period. Males and females who failed to produce a litter as a result of this additional trial period were said to be sterile.

To adjust for body weight in parental and F_1 generations, the organ weights were expressed as mg/100 g of body weight. Student's t test was used to determine if heterosis and/or strain differences were significant. Heterosis was said to have occurred when the F_1 mean was significantly greater (or less) than the mid-parent value. For purposes of analysis, the mid-parent value was assumed to have a weighted average of the parental variances.

In generations F_1–F_4, significant differences in body weights between generations for both males and females were found. Multiple regression analysis (Steel & Torrie, 1960) was used to determine the effects of inbreeding on organ weights and ovulation rate after adjusting for body weight. The regression equation is

$$\hat{Y} = a + b_1 W + b_2 F,$$

where $\hat{Y}$ = organ weight or ovulation rate, a = the Y intercept, W = body weight, F = inbreeding coefficient, and b_1 and b_2 are the partial regression coefficients.

A slightly different model was used for litter size to assess the effect of inbreeding of the parents and of the litter. In a preliminary analysis three independent variables—weight of dam, inbreeding coefficient of dam, and inbreeding coefficient of the litter—were included. Although simple linear regressions showed each to be significant, F tests of the partial regression coefficients indicated that neither the weight of the dam nor the inbreeding of the dam was significant. This was found to be due to the correlation between the dam's weight and inbreeding coefficient, so that if one were included the other was no longer needed. Therefore, the weight of the dam, which was the least significant by itself, was dropped and the final model used was

$$\hat{Y} = a + b_1 F_P + b_2 F_L,$$

where $\hat{Y}$ = litter size, a = the Y intercept, F_P = inbreeding coefficient of the parents, F_L = inbreeding coefficients of the litter, and b_1 and b_2 are the partial regression coefficients.

3. RESULTS

Parental and F_1 organ relative weights (mg/100 g) and evidence of strain differences and heterosis are shown in Table 1. Absolute organ weights of generations F_1–F_4 and the change in weight per 10 % increase in the inbreeding coefficient, F, after adjusting for body weight by regression are shown in Table 2.

For males, significant strain differences existed only for adrenal and testes

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weights. The relative LEW adrenal weights were significantly heavier than CAS adrenals, whereas LEW testes were significantly lighter than CAS.

The only heterotic response found among male organ weights was that of the ventral prostate, which was negative; that is, the mean relative weight of the F_1 prostate was significantly less than both parental strains.

The multiple regression analyses of the inbreeding generations F_1–F_4 indicated that after adjusting for body weight the additional reductions in the weights of the adrenals, seminal vesicles, and ventral prostates due to inbreeding were significant (Table 2). Significant multiple regression equations are shown in Table 3.

Strain differences were found for relative weights of all female organs (Table 1). LEW organs were heavier than CAS organs in all cases. Female pituitary and ovary weights in F_1 hybrids were significantly heavier than the mid-parent value (Table 1) and significant decreases in female thyroid and uterus weights resulted from inbreeding (Table 2).

Table 1. *Strain differences and heterosis in organ weights*

	mg/100 g ± S.E.			Strain difference	Heterosis
	LEW	CAS	F_1	LEW − CAS	F_1 − MP
Thyroid (♂)	7·54 ± 0·10 (72)	7·00 ± 0·44 (13)	7·01 ± 0·17 (30)	+0·54	−0·26
Pituitary (♂)	2·62 ± 0·04 (72)	2·76 ± 0·14 (13)	2·74 ± 0·04 (30)	−0·14	+0·05
Adrenals (♂)	12·69 ± 0·17 (72)	11·34 ± 0·66 (12)	11·91 ± 0·27 (30)	+1·35**	−0·11
Testes	881 ± 9 (71)	939 ± 30 (13)	913 ± 13 (30)	−58*	+3
Seminal vesicles	92 ± 2 (48)	88 ± 6 (10)	94 ± 4 (30)	+4	+4
Ventral prostate	99 ± 2 (48)	103 ± 8 (10)	75 ± 5 (24)	−4	−26**
Body wt. (♂) (g)	324 ± 4 (72)	351 ± 13 (13)	372 ± 8 (30)	.	.
Thyroid (♀)	10·24 ± 0·17 (61)	8·80 ± 0·51 (14)	9·68 ± 0·25 (21)	+1·44**	+0·16
Pituitary (♀)	5·77 ± 0·08 (60)	4·88 ± 0·22 (13)	5·71 ± 0·13 (21)	+0·89**	+0·39*
Adrenals (♀)	26·61 ± 0·33 (60)	22·37 ± 1·72 (14)	25·90 ± 0·55 (21)	+4·24**	+1·41
Ovaries	29·90 ± 0·68 (59)	23·33 ± 2·62 (14)	32·78 ± 0·79 (21)	+6·57**	+6·16**
Uterus	247 ± 6 (61)	197 ± 20 (13)	229 ± 5 (21)	+50**	+7
Body wt. (♀) (g)	202 ± 2 (61)	203 ± 10 (14)	203 ± 3 (21)		

*, ** $P < 0·05$; $P < 0·01$.
Numbers of observations are given in parentheses beneath each mean.
MP represents mid-parent.

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Means and analyses of ovulation rate are presented in Table 4. After adjusting for body weight, strain difference, heterosis, and inbreeding depression were not significant at $P = 0{\cdot}05$.

Table 2. *Organ weights and rates of change due to inbreeding*

	F_1 (mg ± S.E.)	$F_{2,3}$ (mg ± S.E.)	F_4 (mg ± S.E.)	Change due to inbreeding† (mg/10 % F)
Thyroid (♂)	26·1 ± 0·9	25·0 ± 0·6	22·7 ± 1·0	+ 0·06
Pituitary (♂)	10·2 ± 0·5	9·0 ± 0·2	8·4 ± 0·4	− 0·09
Adrenals (♂)	42·0 ± 0·8	36·0 ± 0·9	36·8 ± 1·1	− 0·40*
Testes	3410 ± 57	3270 ± 49	3190 ± 67	+ 2·60
Seminal vesicles	314 ± 13	264 ± 7	240 ± 9	− 5·94**
Ventral prostate	260 ± 18	193 ± 6	202 ± 15	− 6·82*
Body wt. (g) (♂)	377 ± 10	339 ± 7	319 ± 13	.
Thyroid (♀)	20·5 ± 0·5	17·9 ± 0·4	19·3 ± 1·0	− 0·36*
Pituitary (♀)	11·8 ± 0·5	10·7 ± 0·3	11·3 ± 0·4	− 0·16
Adrenals (♀)	51·2 ± 1·2	49·9 ± 1·6	48·9 ± 1·0	− 0·32
Ovaries	64·1 ± 2·6	61·9 ± 1·8	62·5 ± 1·3	− 0·36
Uterus	446 ± 15	407 ± 14	340 ± 17	− 12·26**
Body wt. (g) (♀)	202 ± 5	188 ± 3	198 ± 4	.

Numbers of observations in F_1, $F_{2,3}$ and F_4 were 20, 29 and 13 for males; 13, 34 and 11 for females.

*, ** $P < 0{\cdot}05$, $P < 0{\cdot}01$.

† Change in organ weight due to inbreeding after adjusting for body weight. Values are the partial regression coefficients b_2 divided by 10.

Table 3. *Multiple regression equations*†

Female thyroid	$\hat{Y} = 11{\cdot}15 + 0{\cdot}047W - 3{\cdot}6F$
Male adrenals	$\hat{Y} = 15{\cdot}4 + 0{\cdot}07W - 4{\cdot}0F$
Seminal vesicles	$\hat{Y} = 95 + 0{\cdot}58W - 59{\cdot}4F$
Ventral prostate	$\hat{Y} = 61 + 0{\cdot}52W - 68{\cdot}2F$
Uterus	$\hat{Y} = 179 + 1{\cdot}4W - 123F$

$\hat{Y}$ is estimated organ wt. in mg,; W is body weight in g; and F is inbreeding coefficient.

† Shown only if the additional regression mean square due to F was significant.

All differences in litter size between parental and F_1 matings were significant (Table 5). Since the F_1 litters were from the poor maternal environment of the CAS females, heterosis for litter size occurred in the F_2 litters even though they were 50 % inbred.

To assess the effect of inbreeding of the parents and of the litter on litter size, multiple regression analysis of F_1–F_4 matings was performed. As previously mentioned body weight of the dam was dropped from the model because it was correlated with the inbreeding coefficient of the parents. Thus, the regression due to the inbreeding of the parents and litters includes a portion of the variation in litter size due to body weight. In the analyses of organ weights and ovulation rate that variation due to body weight was removed. When body weight was included in the analysis of litter size the regression equation was

$$\hat{Y} = 12{\cdot}75 + 0{\cdot}018 \text{ dam wt.} - 1{\cdot}5F_P - 9{\cdot}7F_L.$$

The regression coefficients for F_P and F_L differ little from those given in the regression equation in Table 5. Thus, ignoring body weight did not change the results to any great extent. The reduction in litter size due to the inbreeding of the parent was 0·18 pup per 10% increase in F_P. The reduction due to inbreeding of the litter was 1·08 pups per 10% increase in F_L.

Table 4. *Means, standard errors, and analyses of ovulation rate*

	LEW	CAS	F_1	Strain difference LEW − CAS	Heterosis F_1 − MP
No. of observations	31	9	15	.	.
Absolute rate	11·1 ± 0·3	12·2 ± 1·2	12·6 ± 0·5	−1·1	+0·9
Rate/100 g	5·5 ± 0·2	6·3 ± 0·8	6·3 ± 0·3	−0·8	+0·4

	F_1	$F_{2,3}$	F_4	Inbreeding depression†
No. of observations	13	34	11	.
Absolute rate	12·4 ± 0·6	11·9 ± 0·3	11·6 ± 0·5	−0·11/10% F

Regression analysis of ovulation rate (F_1, $F_{2,3}$, F_4)

	D.F.	MS
Regression on body weight	1	23·41**
Additional regression on F	1	3·61
Residual	55	3·09

** $P < 0·01$. † After adjusting for body weight. MP represents mid-parent.

Table 5. *Means, standard errors, and analyses of litter size*

Mating	F_P	F_L	No. of litters	Litter size
LEW × LEW	1	1	57	9·1 ± 0·4
CAS × CAS	1	1	9	3·6 ± 0·7
CAS × LEW	1	0	10	6·6 ± 1·0
$F_1 \times F_1$	0	0·5	18	11·6 ± 0·4
$F_2 \times F_2$	0·5	0·5	31	10·7 ± 0·4
$F_3 \times F_3$	0·5	0·63	12	9·7 ± 0·8
$F_4 \times F_4$	0·63	0·69	15	8·3 ± 0·7

Analysis of variance of parental and F_1 matings

	D.F.	MS
Matings	3	145·32**
Error	90	8·33

Regression analysis of F_1 to F_4 matings

	D.F.	MS
Regression on F_P	1	58·85**
Additional regression on F_L	1	38·88**
Residual	73	5·46

Regression equation: $\hat{Y} = 17·0 - 1·8F_P - 10·8F_L$

F_P is parent's F; F_L is litter's F. ** $P < 0·01$.

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Table 6 summarizes the frequencies of sterility by generation for males and females. A chi-square analysis indicated that CAS males had a significantly higher incidence of sterility than LEW males ($P < 0{\cdot}01$). Although a higher frequency of sterility was found among CAS females than LEW females, the difference was not significant.

In the F_1 generation there were no sterile males or females. Sterility did occur in the F_2 through F_4 generations. The F_2 and F_3 animals had the same inbreeding coefficient but, in males, the frequency of sterility in the F_3 generation was significantly greater than in the F_2 generation ($P < 0{\cdot}05$). Among females, no significant differences between generations F_2 through F_4 were found.

Table 6. *Male and female sterility*

		Males			Females		
Strain or generation	F	No.	No. sterile	% sterile	No.	No. sterile	% sterile
LEW	1	40	6	15	65	13	20
CAS	1	18	13	72	26	9	35
F_1	0	17	0	0	25	0	0
F_2	0·5	35	6	17	41	6	15
F_3	0·5	17	8	47	18	3	17
F_4	0·63	26	12	46	20	4	20

4. DISCUSSION

Jinks & Broadhurst (1963) in a diallel analysis of six strains of rats found that 'determination of litter size is predominantly, if not exclusively, maternal'. In this present study it is clear that litter size is not predominantly maternal as evidenced by the increase in the size of litters born to CAS females upon crossing strains.

The multiple regression analysis and equation for litter size in generations F_1–F_4 (Table 5) is further evidence that both the inbreeding of the parents and of the litter are involved in determination of litter size. Based on litter sizes calculated from the regression equation, the rate of decrease in litter size due to simultaneous inbreeding of parents and litters is 1·25 pups per 10% increase in F.

If it is assumed there were no polyovular follicles and that no monozygotic twins were produced then it could be concluded that the maximum mean litter size could not exceed the mean ovulation rate of the parents. Thus, the maximum mean size of litters born to F_1 females would be 12·6 (the F_1 mean ovulation rate), which is considerably less than the litter size of 17 calculated from the regression equation. This suggests that the regression equation which is based on a rather limited number of F values may be overestimating the effect of inbreeding. If 12·6 is taken as potentially the maximum mean size of litters born to F_1 females then the decrease in litter size due to simultaneous inbreeding of parents and litters would be 0·62 pup per 10% increase in F. This is considerably less than the above-calculated decrease of 1·25 and is similar to the rates of 0·49 and 0·56

reported by Roberts (1960) and Bowman & Falconer (1960), respectively, for mice.

The ovulation rates summarized in Table 4 tempt one to speculate that there was heterosis and inbreeding depression. The values, however, are not significantly different after adjusting for body weight. The only conclusion we can draw from this data is that if body weight declines upon inbreeding, so should the ovulation rate. This was also the conclusion to the study by Falconer & Roberts (1960) with mice.

Some insight into the possible causes of the poor reproductive performance of CAS males and the increasingly poor performance of males in the F_1–F_4 generations may be obtained by considering organ weights. The low-fertility CAS males had larger testes but smaller adrenals than the high-fertility LEW males. Sreebny *et al.* (1959) calculated the mean relative weight of CAS testes and obtained a value almost identical to the one obtained in this study. Significant declines in the weights of the adrenals, seminal vesicles, and ventral prostate indicated inbreeding depression.

The adrenals and testes produce androgen and the seminal vesicles and ventral prostate respond to androgen, suggesting that the cause of the poor reproductive performance in some way involves androgen. The proteolytic activity of the submaxillary gland, which is partially controlled by androgen, is greater in the CAS strain than in the CAR strain, which has smaller testes (Sreebny *et al.* 1957, 1959). Robinson (1965) has suggested that androgen production in the CAS strain could be in excess of normal.

Strain differences were found for all female organ weights, with the LEW strain exceeding the CAS strain in all cases. Female pituitaries and ovaries exhibited heterosis, and the thyroids and uteri exhibited inbreeding depression. There can be little doubt that the small litters born to CAS females and decreasing litter sizes in successive generations of inbreeding were due to endocrine disorders.

An explanation of the extreme differences between the LEW and CAS inbred strains probably lies in the past histories of the two strains. The CAS strain was selected for susceptibility to dental caries. Because of either pleiotropism or linkage, or possibly by coincidence, the CAS strain was simultaneously selected for poor reproductive performance and susceptibility to respiratory infection (Robinson, 1965). In other words, the CAS strain has been selected for poor vigour, suggesting it is homozygous for a number of deleterious recessive alleles. The reproductive performance and vigour of the LEW inbred strain indicates that it is homozygous for a number of beneficial alleles. The heterosis and inbreeding depression observed in this study suggests that these beneficial alleles of the LEW strain show directional dominance.

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