

SPERMATOZOAL HEAD SHAPE IN TWO INBRED STRAINS OF MICE AND THEIR F₁ AND F₂ PROGENIES

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Summary

Seven available inbred strains of mice—A, C57, SWR, C3H, 101, CBA, and DBA—were examined for differences in the shape of their spermatozoal heads. The two most extreme strains with respect to spermatozoal head shape were found to be SWR and C57. The F₁ and F₂ progenies derived from crossing C57 and SWR strains were found to be roughly intermediate between the parent inbred strains. Spermatozoal head shape for these preliminary investigations was calculated as outlined by Penrose (1953). Discriminant analysis was then carried out on F₂ data and a linear discriminant function was obtained whereby 13 characteristics of the spermatozoal head were combined into one "super-character" or discriminant score. The numerical value of the discriminant score was taken as an estimate of spermatozoal head shape for each spermatozoon measured. Analyses of variance carried out on the discriminant scores for each generation revealed that intrastain variation was not significant in the SWR strain and reached only low levels of significance in the C57 strain. The F₁ males were found to be more variable than the inbred males. A large portion of the variability between the F₁ males was shown to arise from "maternal effects". The F₂ males were found to be much more variable than the F₁ males and an estimate of heritability was approximately 0.9. A minimal estimate of the number of "effective factors" operating to distinguish the two inbred parent strains was found to be two. The within-male variance was found not to differ significantly from generation to generation. The implications of these results are discussed.

I. INTRODUCTION

The study of genetic effects on the characteristics of gametes is a relatively new field which has received a certain amount of attention in the last 10 years. Studies have shown that the spermatozoal phenotype is under the control of the animal's genotype as evidenced by significant differences between inbred strains (Braden 1959; Beatty and Napier 1960*b*; Beatty and Sharma 1960); that most morphological spermatozoal characteristics appear to be highly independent of many environmental and biological factors such as weight and litter size (Beatty and Sharma 1960), and of many technical and observational factors (Beatty and Napier 1960*a*); that age trends appear to be unimportant (Beatty and Mukherjee 1963); and that F₁ spermatozoa display heterosis for several characteristics of the head and tail (Sharma 1960).

Interest has also centred on determining whether spermatozoal characteristics are under the control of the diploid soma or the haploid gene complement or both

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(Beatty 1956; Braden 1960; Beatty and Sidhu 1967), and recently an estimate of heritability was reported for midpiece length by Beatty and Woolley (1967), who are now carrying out selection experiments on this characteristic.

The present work is an attempt to ascertain how far the difference in spermatozoal head shape between two inbred strains of mice is heritable and to study the possible nature and relations of the genes determining any heritable differences that are found. The composite characteristic "spermatozoal head shape" was studied rather than individual spermatozoal characters, although these were examined for independent inheritance. Evidence for or against post-meiotic gene action was looked for as well as a search for "maternal effects". Progress reports of the results have been given (Illisson 1965, 1968).

II. MATERIALS AND METHODS

(a) *Experimental Procedure and Sampling*

Seven available inbred strains were examined and the two most extreme strains with respect to spermatozoal head shape were chosen. The seven strains were: A, CBA, DBA, SWR, C57, C3H, and 101, which have all been inbred brother to sister for more than 20 generations at the Division of Animal Genetics, CSIRO. The two extreme strains, namely C57 and SWR, were then crossed and F₁ and F₂ progeny were raised. Weight of the mice was ignored (Beatty and Sharma 1960). Mice younger than 2 months and older than 10 months were not used (Beatty and Mukherjee 1963).

Since the variation between spermatozoa within animals with respect to several characteristics of the spermatozoal head is large and significant (Beatty and Napier 1960a, 1960b; Beatty and Sharma 1960), at least 10 spermatozoa were sampled from each animal.

The techniques employed for determining spermatozoal head shape are rather time-consuming. Therefore an inbred analysis using F₁ and F₂ generations (as outlined by Mather 1949) was adopted rather than an analysis of the correlation between relatives where large numbers of animals have to be studied to gain meaningful results.

(b) *Preparation and Staining*

The mice were killed by stunning and breaking the neck. The tail of the epididymis and the whole of the vas deferens were dissected out and cleaned externally. They were then placed in 0.5 ml of 0.85% NaCl+0.1% glucose [as suggested by Braden (1959) to avoid swelling the acrosome cap] in a cavity slide and the epididymis and vas deferens were teased apart so that the contents of both were emitted into the solution. The resultant suspension was then stirred and two drops were placed on each of three clean slides. Relatively thin smears were made using the edge of another slide and allowed to dry. The smears were then fixed by flooding the slides with methanol for 3 min.

The spermatozoa were stained using a modified version of Hancock's method (described by Bishop and Walton 1960, p. 9) and the procedure outlined by Gurr (1960) for Giemsa stain. The procedure used was as follows: the smears were stained for 20 min in a freshly made mixture of 4 vol. of buffer at pH 11 (1% ethanalamine), 2 vol. of methanol, and 1 vol. of the concentrated Giemsa solution (Gurr 1960). The smears were then washed in the buffer for 5 sec, air-dried, and examined unmounted. Lenzol was found to be the only immersion oil that did not dissolve the stain. This method gives good differential staining of mouse spermatozoa. The acrosome cap is reddish violet in the region where it covers the nucleus while the nucleus itself is pale blue. The limits of both the nucleus and acrosome cap are clearly defined (see Fig. 1). A concurrent study on mouse spermatozoal head morphology as seen with the electron microscope (Illisson 1966) revealed that the acrosome cap covers an area of the nucleus which agrees topographically with the reddish violet region of spermatozoal heads in Giemsa-stained smears.

(c) Methods of Measurement

Spermatozoa were randomly sampled from slides in the following manner: random numbers between 0 and 100 were taken from a table of random numbers and placed in numerical order; a suitable area in the slide was chosen from a quick examination (thick parts of the smear were avoided), and random field No. 1 was designated somewhere in this area. All spermatozoa (usually one or two) that were lying singly in this field were photographed. The slide was then moved along with the mechanical stage to the next field of view (field No. 2), moving so that a lengthwise traverse of the slide was being made. This was continued until the number of the next field corresponded to the next random number in the prepared list of random numbers. In this field, all spermatozoa were photographed as before. This procedure was continued until at least 10 spermatozoa had been photographed.

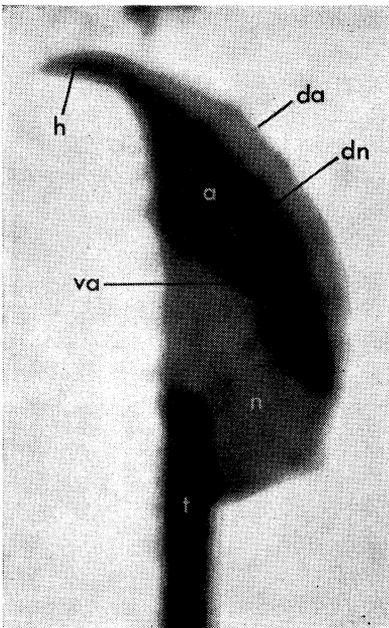


Fig. 1.—Mouse spermatozoal head stained with Giemsa at pH 11. In region *a*, the acrosome cap covers the nucleus; in region *n*, the nucleus is not covered by the acrosome cap. *da*, Dorsal margin of acrosome cap; *dn*, dorsal margin of nucleus; *va*, ventral margin of acrosome cap; *h*, hook; *t*, tail.

The spermatozoa were photographed using a $\times 100$ oil-immersion objective, a $\times 10$ ocular, and fine-grain Kodak Microfilm. Wratten green filter No. 74 was used. Printing was done on Kodak translucent paper and the prints were studied on an X-ray type viewer. The contrast obtained was comparable to that shown in Figure 1. Care was taken to develop the prints so that the background was also seen. In this way the danger of having the true edge of the spermatozoal head obscured by underexposure or underdevelopment was avoided.

Measurements were made with a grid graduated in millimetres that had been printed onto a glass plate. This grid was placed over the prints so that the vertical and horizontal axes of the grid were parallel to the transverse and proximodistal axes of the spermatozoal head (Fig. 2), and the measurements were then read off in turn.

(d) Definition of Spermatozoal Head Shape

The shape of the mouse spermatozoal head was defined at first by 7 arbitrarily chosen measurements parallel to the proximodistal axis of the head and 12 measurements parallel to the transverse axis. These 19 characteristics, called L_1 – L_{10} and W_2 – W_{10} (defined in Fig. 2) were tested for two main possible sources of error. Firstly, there may be present a non-systematic

error likely to be time-dependent in defining the exact limits of the characteristics. Secondly, due to preparative factors, different slides or even different areas within slides could differ significantly with respect to the shape of sperm on them.

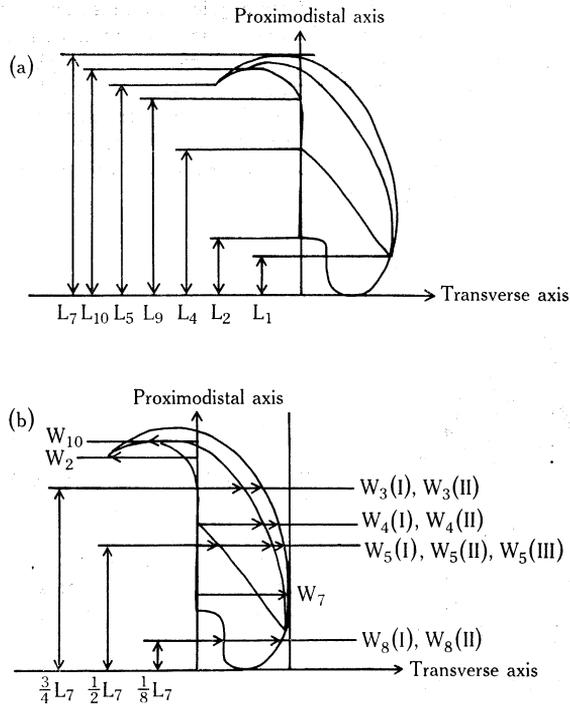


Fig. 2.—Definition of (a) the 7 lengths L_1 – L_{10} and (b) the 12 widths W_2 – W_{10} . All widths extend from the proximidistal axis and are measured parallel to the transverse axis.

- L_1 length to posterior dorsal edge of acrosome cap;
 L_2 length to ventral posterior edge of nucleus;
 L_4 length to ventral posterior edge of acrosome cap;
 L_5 length to tip of hook;
 L_7 maximum length;
 L_9 length to point along proximidistal axis where hook begins to deviate;
 L_{10} length to point of maximum concavity of hook;
 W_2 width to tip of hook;
 W_3 (I) and W_3 (II) width to dorsal edges of nucleus and acrosome cap respectively at a level corresponding to $\frac{3}{4}L_7$;
 W_4 (I) and W_4 (II) width to dorsal edges of nucleus and acrosome cap respectively at level L_4 ;
 W_5 (I), W_5 (II), and W_5 (III) width to ventral edge of acrosome cap and dorsal edges of nucleus and acrosome cap respectively at level $\frac{1}{2}L_7$;
 W_7 maximum width;
 W_8 (I) and W_8 (II) width to ventral and dorsal edges of nucleus respectively at level $\frac{1}{8}L_7$;
 W_{10} width to point of maximum concavity of hook at level L_{10} .

To test the first source of error the same set of spermatozoa were measured on four separate occasions, several months elapsing between each occasion. For the second source of error, spermatozoa from three different slides of the one animal and from three different areas of the one slide were measured.

Analyses of variance revealed that although "slides" and "areas" effects were negligible for all the characteristics and not significant ($P > 0.05$ for 2 and 27 degrees of freedom), a "times" effect was indeed present for the characteristics L_1 , L_5 , W_2 , $W_3(I)$, $W_4(I)$, $W_5(II)$, $W_8(I)$, and W_{10} . Most of these characteristics were, therefore, not used for the definition of spermatozoal head shape. L_1 , $W_3(I)$, and W_{10} were retained since they represent points on the spermatozoal head that are likely to reveal important differences when the category of interest is the variation between classes of animals. Since $W_4(I)$ was discarded, $W_4(II)$ was also discarded. L_{10} was discarded since it was found to be highly correlated with L_7 .

(e) *Methods of Analysis*

For quick comparisons between pairs of groups, a measure of shape difference, called the "shape distance" is calculable from the "coefficient of racial likeness" (CRL) of Pearson (1926), as shown by Penrose (1953). This shape distance has, according to Penrose, the size difference removed from it. Criticism of the CRL and therefore the shape distances of Penrose stems mainly from the fact that the CRL takes no account of the intercorrelations among the variables (Fisher 1936).

A method whereby mutually correlated characteristics may be combined to give a "super character" was given by Mather (1949, p. 31). A similar procedure was used in the present study. Spermatozoal head shape was represented by a linear discriminant function compounded of the 13 characteristics previously defined. The computation was done on a CDC 3600 computer using a programme written by Dr. P. Claringbold (unpublished data). Measurements of 10 spermatozoa from each of 91 F_2 males were used. So that the size factor could be eliminated, all raw measurements were first converted into percentages of the maximum length L_7 . The analysis gives as many linear combinations for the discriminant function as there are variables. The most significant linear combination* ($F = 15.656$ for 90 and 819 degrees of freedom, $P < 0.001$, where $F = \text{ratio of the between-animal mean square for a particular linear combination to the within-animal mean square for the same linear combination}$) gave the discriminant function:

$$X = -0.9 L_1 + 2.4 L_2 + 3.4 L_4 + 2.0 L_9 - 5.7 W_3(I) - 1.9 W_3(II) + 0.7 W_5(I) - 2.5 W_5(II) - 3.0 W_5(III) + 2.1 W_7 - 4.5 W_8(II) - 1.0 W_{10}.$$

The actual X value for each spermatozoon, called the discriminant score, was computed by using the above discriminant function and the raw data as input for the programme Est Score published by Cooley and Lohnes (1962).

III. RESULTS

(a) *The Seven Inbred Strains*

Since neither the head breadth nor head area of spermatozoa differ significantly within inbred strains of mice (Beatty and Sharma 1960) only one male was examined for each of the seven strains. Twenty spermatozoa per male were measured (Table 1) and shape distances between all possible pairs of strains were calculated (Table 2). The significance of the shape distance can be tested since this quantity multiplied by $m[nn'/(n+n')]$ is equivalent to a χ^2 distribution with $(m-1)$ degrees of freedom (Bofinger, personal communication), where $m = \text{number of characteristics}$ and $n = \text{number in sample}$.

The differences between the strains was found to be greatest for the pair C57 and SWR, the shape distance between these two strains being both large and highly

* The F value (15.656) for the most significant linear combination was found to be three times greater than the F value for the next best linear combination. The most significant linear combination could therefore be used on its own with a good degree of confidence (Claringbold, personal communication).

significant ($P < 0.001$ for 12 degrees of freedom). The strain C57 moreover differed markedly from all the other strains in that its spermatozoa were both narrow and very short (see Table 1).

TABLE 1

MEAN SPERMATOZOAL DIMENSIONS OF 13 CHARACTERISTICS IN 7 INBRED STRAINS OF MICE
20 spermatozoa of one male from each strain were measured. The common standard deviation was calculated by pooling the individual variances of each strain and taking the mean. This is valid provided the variances of the 7 strains are homogeneous. Most of the characteristics were found to show homogeneity of variance but the sampling (20) was small and in general homogeneity of variance was assumed

Charac- teristic*	$(6 \times 10^3) \times$ Mean Measurement (mm) of Spermatozoal Head Characteristic in the Strain:							Common Standard Deviation
	C57	SWR	CBA	DBA	C3H	101	A	
L ₁	9.3	10.0	10.7	9.6	11.0	9.7	8.6	1.06
L ₂	9.1	12.2	13.1	13.4	12.7	12.1	13.1	0.81
L ₄	24.7	28.9	30.3	31.9	30.3	28.2	29.8	0.86
L ₇	43.4	49.5	50.5	50.9	49.1	46.9	48.5	1.29
L ₉	33.2	39.0	41.4	40.6	38.6	36.7	37.4	1.37
W ₃ (I)	9.9	9.0	11.1	12.4	10.8	9.7	10.9	0.73
W ₃ (II)	13.4	12.7	15.0	16.4	15.5	14.2	15.0	0.73
W ₅ (I)	5.1	5.3	8.6	9.2	8.7	6.5	8.3	0.93
W ₅ (II)	16.1	16.5	17.8	19.1	18.4	17.0	18.6	0.70
W ₅ (III)	18.3	18.8	20.2	21.5	21.0	19.7	20.9	0.57
W ₇	19.5	20.1	20.9	22.5	22.0	21.1	22.2	0.71
W ₈ (II)	17.6	18.4	17.9	20.3	18.9	19.6	19.2	0.88
W ₁₀	8.8	9.0	8.4	9.0	8.9	10.1	9.7	0.74

* As defined in Figure 2.

TABLE 2

SHAPE DISTANCES BETWEEN ALL POSSIBLE PAIRS OF THE 7 INBRED STRAINS

Strain	Strain					
	CBA	DBA	C3H	SWR	C57	101
A	23.7	8.9	7.6	37.3	37.4	13.2
CBA		16.0	6.2	19.1	58.3	36.1
DBA			8.5	37.4	62.1	28.4
C3H				30.1	36.4	15.8
SWR					74.5	20.8
C57						17.1

(b) *Strains C57 and SWR*

The mean values for each characteristic for the strains C57 and SWR are shown in Table 3. They were taken from eight litters, one male per litter, and 10 spermatozoa per male. Significant differences between all characteristics except L₁, W₅(I), and

TABLE 3
 MEANS AND STANDARD DEVIATIONS OF SPERMATOZOAL HEAD CHARACTERISTICS OF PARENTS AND F₁ AND F₂ PROGENIES, AND DIFFERENCES BETWEEN THESE GENERATIONS AND THE MID-PARENT VALUES (MP)
 Values are expressed in millimetres, and are (5.8 × 10³) times the actual value

Charac- teristic	C57 Parent		SWR Parent		F ₁ Progeny		F ₂ Progeny		Difference Between Means†				
	Mean (n = 80)	S.D.	Mean (n = 80)	S.D.	Mean (n = 330)	S.D.	Mean (n = 910)	S.D.	SWR-C57	F ₁ -MP	F ₂ -MP	F ₁ -MP	F ₂ -MP
L ₁	9.23	1.239	9.50	1.060	9.22	1.032	9.25	1.248	+0.27	-0.14**	-0.11**	-0.14**	-0.11**
L ₂	8.98	1.014	12.23	1.060	11.02	0.869	10.89	1.197	+3.25**	+0.42**	+0.29**	+0.42**	+0.29**
L ₄	24.58	1.190	27.98	1.408	26.82	1.327	26.97	1.945	+3.40**	+0.54**	+0.69**	+0.54**	+0.69**
L ₇	41.97	1.376	47.44	1.651	45.71	1.459	45.51	2.214	+5.47**	+1.00**	+0.80**	+1.00**	+0.80**
L ₉	32.04	1.855	37.82	2.056	35.53	1.826	35.19	2.677	+5.78**	+0.60**	+0.26**	+0.60**	+0.26**
W ₃ (I)	9.91	0.800	8.26	0.891	8.54	0.832	8.58	0.895	-1.65**	-0.54**	-0.50**	-0.54**	-0.50**
W ₃ (II)	13.38	0.851	12.12	0.703	13.02	0.732	13.00	0.803	-1.26**	+0.27**	+0.25**	+0.27**	+0.25**
W ₅ (I)	5.95	1.099	5.68	1.138	5.66	1.044	5.91	1.394	-0.27	-0.16**	+0.09	-0.16**	+0.09
W ₅ (II)	15.95	0.893	15.45	0.942	15.56	0.752	15.61	0.882	-0.50**	-0.14**	-0.09	-0.14**	-0.09
W ₅ (III)	17.92	0.761	17.70	0.733	17.88	0.716	17.99	0.787	-0.22*	+0.08	+0.19**	+0.08	+0.19**
W ₅	18.98	0.829	18.93	0.884	18.88	0.749	19.15	0.849	-0.05	-0.08	+0.19**	-0.05	+0.19**
W ₈ (II)	16.78	1.220	17.16	1.104	16.62	0.888	16.95	0.949	+0.38*	-0.35**	-0.02	+0.38*	-0.02
W ₁₀	7.96	0.788	8.32	0.932	8.35	0.689	8.69	0.966	+0.36**	-0.21**	+0.55**	-0.21**	+0.55**

* 0.01 < P < 0.05. ** P < 0.001. † Significance of differences tested using Student's t-test.

W_7 were found (Table 3). C57 spermatozoa have markedly shorter heads than SWR spermatozoa (L_7), although the spermatozoa from both strains have similar maximum widths (W_7). This leads to a marked shape difference. Also, the SWR spermatozoal heads taper much more rapidly before curving over to form the hook, thus giving relatively low values to the characteristics $W_3(I)$ and $W_3(II)$.

The shape distance between the strains was found to be 70.1 which is highly significant ($P < 0.001$, 12 degrees of freedom) and compares well with the value obtained in the limited comparison in the initial experiment (Table 2). The variances between all corresponding characteristics in the two strains were found to be homogeneous when the variance ratio test was applied.

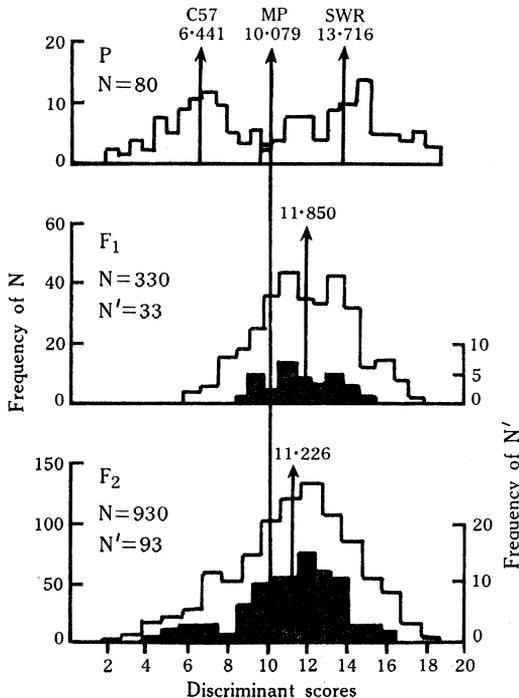


Fig. 3.—Frequency distributions of the discriminant scores of the spermatozoa and the male means for the parent (P), F_1 , and F_2 generations. The open histograms represent the frequency distributions of the N spermatozoa in each generation. The solid histograms represent the frequency distributions of the means of the N' males in each generation. Means for each generation are indicated as well as the mid-parent mean (MP).

Intrastrain variation within the strains C57 and SWR was analysed using the computed discriminant scores and an analysis of variance. The actual frequency distributions of the discriminant scores (Fig. 3) were first tested for goodness of fit with theoretical normal distributions. The discriminant scores were normally distributed with $\chi^2 = 11.21$ with 9 degrees of freedom for the C57 strain and 9.74 with 9 degrees of freedom for the SWR strain.

The analysis of variance (Snedecor 1956) of the two strains (Table 4) showed that the difference between the two strains C57 and SWR is highly significant. This result using discriminant scores agrees well with the results obtained using shape distances. The males-in-strains effect reached low levels of significance. However, the between-males variance component is low, comprising only 2% of the total variation (or one-seventh of the total within-strains variance), whereas the sperm-in-males variance component is much larger.

(c) F_1 and F_2 Generations

Table 3 gives the mean values of the spermatozoal characteristics of 33 F_1 male progeny derived from crossing the C57 and SWR strains. Differences between F_1 and mid-parent means exist mainly along the length (or proximodistal) axis, where four out of five values are significantly greater in the F_1 than in the mid-parent. Along the width axis, five out of eight values in the F_1 are less than in the mid-parent; i.e. the F_1 spermatozoa appear to resemble the longer and narrower SWR parent spermatozoa more than the shorter and wider C57 spermatozoa.

TABLE 4

ANALYSIS OF VARIANCE OF THE INBRED STRAINS C57 AND SWR USING DISCRIMINANT SCORES
The analysis is based on the assumptions that the within-male and between-male mean squares are of the same order in both strains. Analyses of variance conducted on the two strains separately revealed that these assumptions were justified. The males-in-strains mean squares [ms(MI)] as well as the sperm-in-males mean squares [ms(SM)] were found not to differ significantly between the two strains. Significance tests gave the following results, where ms(I) represents the strains mean square: strains, $F = \text{ms(I)}/\text{ms(MI)} = 242.5$, $P \ll 0.001$; males-in-strains, $F = \text{ms(MI)}/\text{ms(SM)} = 2.31$, $0.01 < P < 0.05$

Source of Variation	Degrees of Freedom	Mean Square	Parameters	Variance Components	Components as % of Total Variance
Strains	1	2116.981	$\sigma^2 + n\sigma_B^2 + bn\sigma_A^2$ *	26.400	86
Males-in-strains	14	8.731	$\sigma^2 + n\sigma_B^2$	0.495	2
Sperm-in-males	144	3.781	σ^2	3.781	12
Total	159			30.676	

* $b = 8$; $n = 10$.

The deviations of the F_2 values from the mid-parent are generally similar to but less than the deviations of the F_1 values (Table 3). Seven of the 13 characteristics in the F_2 lie between the F_1 and mid-parent [L_1 , L_2 , L_7 , W_3 (I), W_3 (II), and W_8 (II)]. Two of the characteristics (L_4 and W_{10}) are unusual in that they deviate more from the mid-parent in the F_2 than they do in the F_1 . The rest of the characteristics [W_5 (I), W_5 (III), and W_7] do not show any obvious trends, a feature that is to be expected since the differences themselves are small and in some cases not significant.

The frequency distributions of the discriminant scores of the F_1 and F_2 generations (Fig. 3) were tested for goodness of fit with theoretical normal distributions. For the F_1 generation, the scores for the 330 spermatozoa were found to be normally distributed, with $\chi^2 = 13.04$ with 13 degrees of freedom ($P = 0.40$). For the F_2 generation, the mean scores of the 91 males were also normally distributed, with $\chi^2 = 9.07$ with 9 degrees of freedom ($P = 0.40$).

The mean discriminant score for the F_1 generation (11.850) lies on the SWR side of the mid-parent value (Fig. 3), thus agreeing with trends noted when individual characteristics (Table 3) were examined. The mean discriminant score for the F_2 generation (11.226) lies between the F_1 and the mid-parent values (Fig. 3). Indications

that the shape of the F_2 spermatozoa is intermediate between the F_1 and mid-parent spermatozoa were also obtained earlier (Table 3).

(d) *Analyses of Variance of the F_1 and F_2 Discriminant Scores*

The analysis of variance of the discriminant scores of the 330 F_1 spermatozoa (Table 5) was based on 13 litters, unequal numbers of males per litter, and 10 spermatozoa per male. The analysis of variance of the discriminant scores of the 910 F_2 spermatozoa (Table 5) was based on 26 litters, unequal numbers of males per litter, and 10 spermatozoa per male.

TABLE 5
ANALYSES OF VARIANCE OF THE F_1 AND F_2 GENERATIONS USING DISCRIMINANT SCORES

Source of Variation	F_1 Generation			F_2 Generation		
	D.F.	Mean Square	Variance Component	D.F.	Mean Square	Variance Component
Males	32	29.774	2.634	90	61.065	5.654
Litters	12	54.089	1.562	25	90.111	1.155
Males-in-litters	20	15.186	1.175	65	49.894	4.537
Sperm-in-males	297	3.432	3.432	819	4.521	4.521
Total	329		6.066	909		10.175

The F_1 males differed significantly ($F = 8.68$, $P < 0.001$), and the between-males variance component (2.634) was substantially higher than that found in the parents (0.495, Table 4). The variability between the F_1 males arose both from significant differences between litters ($F = 3.562$; $0.001 < P < 0.01$), the variability of which could partly stem from "maternal effects" since reciprocal matings were carried out, and from significant differences between males within litters. The existence of maternal effects was demonstrated by a suitable analysis of variance (Table 6). Two mating types (direct and reciprocal), 11 matings (one litter per mating), and 31 males were used.

The mating-type effect was highly significant ($F = 22.3$, $P < 0.001$) and the mating-type variance component was responsible for 63% of the total variation seen between F_1 males (Table 6). Thus, the F_1 males derived from direct matings ($C57\text{♀} \times \text{SWR}\text{♂}$) are significantly different from the males derived from reciprocal matings ($C57\text{♂} \times \text{SWR}\text{♀}$), and the differences between litters of the same mating type are relatively negligible (3% of the total variation).

However, when litter effects (maternal effects) are removed, the variation left (males-in-litters) is still large (1.175, Table 5), larger even than the between-litters variation seen in the parent inbred strains (0.495, Table 4).

Spermatozoa-within-males were no more variable in the F_1 than the parent males, as evidenced from the similar sperm-in-males variance components (3.432 for F_1 , Table 5; and 3.781 for parents, Table 4).

The F_2 males differed very significantly from one another ($F = 13.5$, $P < 0.001$), whereas the difference between litters reached only low levels of significance ($F = 1.81$,

$0.01 < P < 0.05$; Table 5). The between-males variance component was markedly greater than the corresponding variance components in the F_1 (excluding litter effects, 4.537 cf. 1.175) or in the parents (0.495).

The spermatozoa-within-males variance component in the F_2 (4.521) is slightly greater than, although of the same order as, that in the parental and F_1 generations.

TABLE 6
ANALYSIS OF VARIANCE OF F_1 MALES

Source of Variation	D.F.	Mean Square	Variance Component	Components as % of Total Variance
Mating type	1	43.665	2.753	63
Litters-within-types	9	1.960	0.161	3
Males-in-litters	20	1.521	1.521	34

(e) *Genetic Variance in the F_2*

The F_2 phenotypic variance may be separated into components thus:

$$V_P = V_G + V_E$$

where V_P = total phenotypic variance, V_G = total genetic variance, and V_E = total environmental variance. A direct measure of V_E is the variance seen in the parent inbred strains and in the F_1 progeny with the following assumptions:

- (1) The environmental variance is the same in all genotypes. This certainly is not always true (Lerner 1954).
- (2) The variance in inbred lines is all environmental in origin. However, some of the variance may be genetic due to residual heterozygosity that may be present because of natural selection.
- (3) The total variance seen in the F_1 generation is only environmental in origin.

The total variance in the F_1 generation may include a genetic component if gene action on spermatozoal head shape occurs after the meiotic divisions. Some evidence has already been presented which suggests that gene action on spermatozoal head shape does not occur in the haploid spermatozoon after the meiotic divisions.

The environmental variance may be subdivided into a within-males variance, V_{Es} , and a between-males variance, V_{Eg} . V_{Es} presumably reflects the effects of technique, observation, and natural variation between spermatozoa. This natural variation probably arises from localized circumstances operating during the spermatozoon's development. Since spermatozoa are produced continuously, a batch of spermatozoa produced at one time may differ from a batch produced at another time. Some evidence that this does not occur was given by Beatty and Napier (1960a).

A summary of the between-male ($V_{Eg} + V_G$), within-male (V_{Es}), and total phenotypic (V_P) components of variance for the parent, F_1 , and F_2 generations is given in Table 7. For the calculation of the genetic variance V_G in the F_2 generation, we have:

$$V_P = V_G + V_E,$$

where

$$V_E = \frac{1}{3}[V_P(\text{C57}) + V_P(\text{SWR}) + V_P(\text{F}_1)].$$

Therefore

$$V_G = 10 \cdot 175 - \frac{1}{3}(3 \cdot 908 + 4 \cdot 645 + 6 \cdot 066) = 5 \cdot 302.$$

TABLE 7
VARIANCE COMPONENTS AND MEAN DISCRIMINANT SCORES OF THE PARENTAL, F_1 , AND F_2 GENERATIONS

Variance components are taken from the respective analyses of variance shown in Tables 4 and 5

Generation	Variance Components				Mean Discriminant Score
	Between-males ($V_G + V_{Eg}$)*	S.D. of ($V_G + V_{Eg}$)	Within-males (V_{Es})	Total Phenotypic (V_P)	
C57 parent	0.402	0.477	3.506	3.908	6.441
SWR parent	0.588		4.057	4.645	13.716
F_1	2.634		3.432	6.066	11.850
F_2	5.654	0.901	4.521	10.175	11.226

* It is assumed that V_G is negligible in the parent and F_1 generations.

Since 10 spermatozoa were measured from each animal, there is a gain in accuracy which is seen as a reduction in the value of V_P thus (Falconer 1961, p. 146):

$$V_P(n) = V_G + V_{Eg} + V_{Es}/n,$$

where n = number of determinations per animal. Therefore

$$V_P(n) = 5.654 + 4.521/10 = 6.106.$$

An estimate of heritability (h^2) can then be obtained:

$$\begin{aligned} h^2 &= V_G/V_P(n) \\ &= 5.302/6.106 = 0.87 \simeq 0.9. \end{aligned}$$

A discussion of the errors involved in the determination of V_G must of course begin with a discussion of the sampling errors of the components that were used to calculate it, since there is no standard way of calculating standard deviations for V_G or the ratio V_G/V_P directly. The standard deviation of ($V_G + V_{Eg}$) obtained from the F_2 was found to be 0.901 (Table 7) and that of V_{Eg} obtained from the parents and F_1 was found to be 0.477 (after Snedecor 1956, p. 261). Roughly, this means that the standard deviation of V_G does not exceed 1.4.

Knowledge of V_G in the F_2 generation allows an approximate estimate to be made of the number (k) of "effective factors" (Mather 1949). A minimal estimate K_1 of k is given by

$$K_1 = (\bar{P}_1 - \bar{P}_2)^2 / 4D,$$

where $D = 2V_G(F_2)$ and \bar{P}_1 and \bar{P}_2 are means of the SWR and C57 parental generations respectively. Therefore

$$K_1 = (13.716 - 6.441)^2 / 8(5.302) \simeq 2.$$

The approximate limits of K_1 could not be calculated from the data available and all that can be concluded from the value obtained is that the number of effective factors is closer to 2 than to (say) 20.

(f) Individual Characteristics

The individual characteristics of the spermatozoal head were also examined to determine the extent to which they may be jointly heritable. Genetic correlation coefficients were calculated as described by Kempthorne (1957) for selected pairs of length and width characteristics using F_2 data (910 readings) as well as pooled parental data (160 readings) (Table 8).

TABLE 8
"GENETIC" AND "ERROR" CORRELATIONS FOR SEVERAL PAIRS OF CHARACTERISTICS OF THE SPERMATOZOAL HEAD IN THE F_2 AND PARENTAL GENERATIONS

Pair of Characters	Genetic Correlation		Error Correlation	
	Parents ($n = 160$)	F_2 ($n = 910$)	Parents ($n = 160$)	F_2 ($n = 910$)
L_2 and L_7	0.87	0.83	0.59	0.59
L_4 and L_7	0.98	0.93	0.72	0.71
L_7 and W_7	0.07	0.34	0.17	0.08
W_7 and $W_8(\text{II})$	0.82	0.74	0.69	0.63
$W_3(\text{I})$ and $W_5(\text{II})$	0.75	0.72	0.56	0.53

The genetic correlation coefficients for pairs of length characteristics (L_2 and L_7 , L_4 and L_7) were high in the parents and F_2 , while the genetic correlation coefficients between pairs of width characteristics [W_7 and $W_8(\text{II})$, $W_3(\text{I})$ and $W_5(\text{II})$] were also high in both generations. The parental and F_2 correlations were in all these cases obviously not significantly different, although significance tests could not be applied (Kempthorne 1957). When the maximum length (L_7) was correlated with the maximum width (W_7), however, the genetic correlation coefficient was much lower both in the parental and F_2 generations.

IV. DISCUSSION

(a) Strain Differences in Spermatozoal Head Shape

Beatty and Sharma (1960) found that several characteristics of spermatozoa including the breadth and area of the head vary significantly among inbred strains of mice. In the present study, not isolated characteristics but the shape of the

spermatozoal head as a whole was shown to vary, in some cases significantly, between inbred strains of mice. This is direct evidence that the spermatozoal head shape is visibly affected by the genotype of the animal. The largest differences were found between the strains C57 and SWR, C57 and CBA, and C57 and DBA. The strain C57 was outstanding in that the spermatozoa were relatively small and differed greatly in shape from most of the other strains studied. Beatty and Sharma (1960) also found that six out of eight strains were larger and significantly different from the C57 strain with respect to spermatozoal head breadth. Particularly, the head breadth of A-strain spermatozoa was shown to be 10% greater than that of C57-strain spermatozoa. In the present study the maximum width of the spermatozoa of A-strain males was found to be approximately 15% greater than the corresponding characteristic in the spermatozoa of C57 males. Also, the narrowness of live C57 spermatozoa compared with live CBA spermatozoa was reported by Braden (1959) and by Beatty and Sharma (1960). This was also seen in Giemsa preparations in the present study. It is thus evident that the methods of preparation have not altered the true dimensions of the spermatozoa.

(b) Variation in the Inbred Strains C57 and SWR and Their F₁ Progeny

Using discriminant scores as a measure of spermatozoal head shape, it was found that the mean variance of the males in the strains C57 and SWR was 0.495 while the variance of the F₁ males was 2.634. It is therefore apparent that inbreeding has not led to the increase in variability that has been reported for many quantitative characters (reviewed by Lerner 1954). Indeed, the F₁ between-male variance was found actually to be significantly greater than that of the inbred strains. A large portion of the increase in variability of the F₁ males over the inbred males was, however, seen to stem from litter differences which were seen to arise mainly from maternal effects.

The homogeneity of the males in the strains C57 and SWR implies that factors such as age, weight, and other environmental factors have little effect in altering the mean spermatozoal head shape. This homogeneity was also reported by Beatty and Sharma (1960) for several characteristics of spermatozoa. However, the number of abnormal spermatozoa increase with age and Beatty and Mukherjee (1963) also reported that the head breadth of spermatozoa is affected by extremes of age.

Although the variability between males within the inbred strains was found to be small, the variation of spermatozoa within individual males was relatively large, constituting about 90% of the total phenotypic variation seen in the inbred strains. The within-males variance was 4.057 in the SWR strain, 3.506 in the C57 strain, and 3.432 in the F₁. The within-animal variation did not differ significantly between the inbred strains and the F₁.

It thus appears that the variation arising from technique and uncertainties of development is constant throughout the inbred strains and the F₁ and is much greater than the variation that arises from the effects of environmental factors that cause animals to differ from one another. The present results on spermatozoal head shape agree with those of Beatty and Napier (1960*b*) for head length of rabbit spermatozoa and with those of Beatty and Sharma (1960) for head breadth and head area of

mouse spermatozoa. Very little work has been done on other characteristics to see whether this phenomenon is a general one. Measurements of bristle number on different segments in *Drosophila melanogaster* showed that 97% of the environmental variation stemmed from variation within animals (Reeve and Robertson 1954). Also Robertson (1957) showed that 91% of the environmental variation in ovary size in *Drosophila melanogaster* stemmed from the variation within animals. This phenomenon may prove to be a common one in the animal kingdom.

The spermatozoa in inbred males are all alike genetically whereas those of F_1 males are diverse as a result of segregation of chromosomes during the meiotic divisions. Hence if gene action were exerted in the developing haploid spermatid, the spermatozoa might be expected to develop along different pathways giving rise to a more variable population of mature spermatozoa than seen in the inbred strains. Sharma (1960) gave some evidence that the variance of F_1 males from six crosses between four inbred strains was of the same order as seen in the inbred strains. In the present study, the within-animal variance in the F_1 was of the same order as (actually slightly less than) that in the parent strains. Therefore Sharma's suggestion that post-meiotic gene action does not visibly affect the mensuration characteristics of spermatozoa is supported by the present study. Beatty and Sidhu (1967) produced evidence that also in *Drosophila* "haploid effects" are not present as judged from a comparison of $M/+$ males with $+/+$ males (M = the gene *minute*).

With regard to maternal effects that are commonly seen for quantitative characters, the reciprocal and direct males were seen to deviate towards the spermatozoal type typical of the female parent strain. The difference between reciprocal and direct males was found to be highly significant, and was a major factor in causing the significance of the between-litter mean square in the F_1 . The present study is the first demonstration of significant maternal effects on a spermatozoal characteristic, viz. head shape. These maternal effects must have a permanent influence on the indifferent germ cells or the Sertoli cells of the embryo, since the effects appear to be transmitted to all future generations of spermatozoa.

The F_1 mean discriminant score (11.850) deviated significantly from the mid-parent value and lay between the mid-parent and SWR mean values (10.079 and 13.716 respectively). Therefore dominance of the SWR parent set of genes acting in the same direction was found to be present. The SWR parent moreover has markedly longer spermatozoa than the C57 parent. The deviation of the F_1 is thus towards the longer spermatozoal type. These results agree with those of Sharma (1960) who observed positive heterosis for spermatozoal head area but not for head breadth.

(c) Variation in the F_2 Progeny

The F_2 mean discriminant score was found to be 11.220, which is intermediate between the F_1 and the mid-parent values. Theoretically, C should equal 0 where $C = 4\bar{F}_2 - 2\bar{F}_1 - \bar{P}_1 - \bar{P}_2$ within the limits of sampling error (Mather 1949), so that standard error of $C = [16V(\bar{F}_2) + 4V(F_1) + V(P_1) + V(\bar{P}_2)]^{\frac{1}{2}}$, where \bar{F}_2 , \bar{F}_1 , \bar{P}_1 , and \bar{P}_2 represent the mean value for the F_2 , F_1 , and the two parent generations and $V(\bar{F}_2)$, $V(\bar{F}_1)$, $V(\bar{P}_1)$, and $V(\bar{P}_2)$ represent the total variance in the F_2 , F_1 , and the two parent generations respectively. It was found that $C = 1.047 \pm 10.1$. Thus it

can be seen that the actual F_2 mean agrees well with the theoretical F_2 mean. The value of C moreover can be used to test the adequacy of the scale (Mather 1949). Thus there is also some evidence that the scale requirements are satisfied by the discriminant scores.

The between-males variance of the F_2 increased to 5.654 (Table 7) indicating that segregation had occurred. By Mather's formula (Mather 1949), and with certain simplifying assumptions quoted by Mather, the minimal number of effective factors acting on spermatozoal head shape was found to be two. This probably means that there are at least two segments of chromosomes, acting as units of inheritance, that contain genes that are responsible for controlling the shape of spermatozoa in C57 and SWR strains.

When the characteristics of the spermatozoal head were considered individually, it was seen that most of the characteristics behaved similarly in the F_2 , that is, their mean F_2 values lay somewhere between the F_1 and mid-parent values. One of the characteristics, namely the length to the ventral posterior edge of the acrosome cap, L_4 , behaved differently in that its mean F_2 value deviated more from the mid-parent than did the F_1 value. This result could perhaps mean that the characteristics of the spermatozoal head are under the control of different genes. The rather small genetic correlation seen between maximum length and width in the F_2 and parental generations (Table 8) could also be taken as evidence of independent inheritance for these two characteristics. However, the high genetic correlations obtained for pairs of length and pairs of width characteristics suggest that a high extent of common genetic control is present.

(d) Heritability of Spermatozoal Head Shape

The degree of genetic determination of spermatozoal head shape, estimated as the ratio of the total genetic to the total phenotypic variance, was taken to be heritability in the broad sense. It was high ($h^2 \simeq 0.9$) in the F_2 generation obtained from crossing C57 and SWR strains. A strong genetic determination means that the effects of environment are small in comparison. A high heritability (0.97 ± 0.36) was also obtained by Beatty and Woolley (1967) for midpiece length in the mouse. High heritability values for spermatozoal mensuration characteristics can be expected to exist since these characteristics probably do not act as important determinants for reproductive fitness. Therefore, it is not surprising that spermatozoal head shape is a conservative characteristic at least within classes of animals. Its use as a taxonomic criterion is being investigated.

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