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Selection for the length of the spermatozoan midpiece in the mouse

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SUMMARY

A subcellular dimension, the length of the spermatozoan midpiece, has been the subject of a two-way selection programme in mice. After thirteen generations of within-family selection the divergence between the selected lines amounted to 5·4 phenotypic standard deviations. The realized heritability was found to be $0{\cdot}76 \pm 0{\cdot}02$, dominance and common-environmental effects being apparently very small. The reality of the selection response was verified in measurements on live spermatozoa. Other measurements have shown that the change in the length of the midpiece has been independent of its width, and has occurred without a proportionate change in the length of the main-piece of the flagellum. An electron microscope study has shown that the selection has, in fact, brought about changes in the quantity of mitochondrial material in the sperm cells.

1. INTRODUCTION

An early report of statistically significant differences between individual males in the morphology of their spermatozoa was that of Friend (1936), who discovered differences in the length of the spermatozoan head and tail in several rodent species. More recently, Braden (1956, 1959) found that inbred strains of mice differed from one another in the shape of the spermatozoan nucleus; Beatty & Napier (1960) demonstrated marked differences between strains of rabbit in the dimensions of the spermatozoan head; and Napier (1961) furthered these studies by estimating the heritabilities of six spermatozoan characters from son–sire regressions. His general conclusion was that mensuration characters, such as head length and breadth, have heritabilities of about 0·7, whereas, for characters more obviously related to reproductive fitness, for example, the percentage of capless spermatozoa, the heritability is very low. Following Braden's initial work on the mouse spermatozoon, Beatty & Sharma (1960) compared eight inbred strains and were able to demonstrate differences in all the dimensional characters studied except the projected area of the midpiece. They concluded, from the lack of appreciable variation between litters-in-strains, males-in-litters, and preparations-in-males, that the dimensions of the spermatozoa were remarkably independent of environmental and technical sources of variation. There was always a relatively large and unexplained variance component for spermatozoa within preparations,

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as had been observed in the studies on rabbit spermatozoa already mentioned. Sharma (1960) later confirmed the results obtained for four of these inbred strains, and found that the spermatozoan characteristics of the F_1 crosses were, in general, intermediate between the parent strains. Corroboration of these findings has recently come from another laboratory in a paper by Illisson (1969), who has studied the spermatozoan 'head shape' of the mouse, a composite character defined by a linear discriminant function. Her findings confirm the general pattern of variation to be found in the dimensions of spermatozoa, and the magnitude of its genetic components. In addition, she estimated, from an analysis of the variation in an F_2 generation, that the distinction between the character 'head shape' in the two parent strains (SWR and C57) is controlled by a minimum of approximately two 'effective factors'. The heritability of the character was found to be about 0·9. Beatty (1961, 1970) has reviewed the evidence for the genetic influences on the phenotype of the mammalian gamete.

Since it has been shown that the dimensional characteristics of spermatozoa are highly heritable, they would be expected to respond rapidly to artificial selection. This has been tested in the present work. Apart from simply investigating whether it is possible to produce changes in spermatozoa in this way, the major interest in such a selection experiment is that, by exaggerating the variation initially present in the population, new experimental material would be created which subsequently could be used in investigating the extent to which the character has an adaptive value in terms of the function of the cell. The character studied—the length of the midpiece of the mouse spermatozoon—was chosen because it was thought to have a fairly high heritability (Woolley & Beatty, 1967), and also because of the relative ease and accuracy with which it can be measured. Further, since the midpiece is composed largely of mitochondria, it was felt that a change in its dimensions might have an effect on the motility or general vitality of the cell. During the course of the selection experiment, a record was kept of the reproductive performance of the mice. Later in the selection programme, when a response seemed to have been established, verification of the response was sought from measurements of live spermatozoa; the selected lines were examined for changes in the width of the midpiece; and the possibility of correlated changes in other spermatozoan dimensions was also examined. Finally, the analysis of the response to selection was concluded with a study of the arrangement of the midpiece mitochondria in the selected and control lines.

2. MATERIAL AND METHODS

Selection

The mice used in the selection experiment were from a stock of mixed origin, the Edinburgh 'Q' strain. This strain was established in 1957 from crosses between five non-inbred and one inbred strain, and subsequently had been maintained with minimal inbreeding. Prior to the start of the present work, the 'Q' strain had been bred as six separate lines for five generations. The mice used in the selection programme were drawn almost equally from five of the lines.

Spermatozoa were obtained from the mice by killing the males and removing the sperm from the vasa deferentia. Two possible alternative methods were considered, that of electro-ejaculation, recently described by Snyder (1966), and the retrieval of spermatozoa *post coitum* from the female genital tract. The former method was rejected as being rather hazardous and of unproven reliability; the latter on account of the labour involved and the additional variables introduced. Therefore, since the males were not scored for their midpiece length while alive, progeny had to be reared from them beforehand and selection made retrospectively on the litters. This meant that most of the cage space was occupied by litters which were eventually to be discarded, and the effective population size was thus less than would otherwise have been possible. This restriction on population size influenced the choice of a within-family selection programme, which would maintain a comparatively large effective population. Selection for an increase in the length of the spermatozoan midpiece was made in one line (high line), and selection for a decreased length in another line (low line). A third line was maintained in exactly the same way but the selection of the male parents was made at random (control line). Each line consisted of eight full-sib families per generation.

The base population was obtained by choosing eight litters, at least one from each of the five available 'Q' lines; the largest litters which included at least three sibs of each sex were chosen. These eight sibships, numbered 1–8, were then crossed reciprocally (i.e. 1♀♀ × 2♂♂, 2♀♀ × 1♂♂; 3♀♀ × 4♂♂, 4♀♀ × 3♂♂, etc.), giving a total of 32 matings. The 32 male parents were killed after being caged for 14 days with the females and smears were made of their spermatozoa. The midpiece length was scored for each male and then, within each family, the males were assigned to the high or low line on this basis. An intermediate male from each litter was assigned to the control line. The litters which had been sired by these mice were reared, and, within each line, the offspring were mated reciprocally between sibships at 6–7 weeks of age. The males were killed after 2 weeks of access to the females, smears were made of their spermatozoa, and after the midpiece length had been scored, selection was made within sibships. Subsequently, the matings followed a cyclical pattern designed to minimize inbreeding: after thirteen generations under this system the coefficient of inbreeding (ignoring the rare irregularities of procedure due to sterility, etc.) was approximately 17 % (computed by D. S. Falconer).

The midpiece length 'score' for a male was always the mean length for ten spermatozoa in observation units. The selection differential on males was calculated as the mean deviation of the selected males from their family means. The net selection differential was half this, since the females were unselected. In the event of there being, through infertility or an unbalanced sex ratio, no mice available for a family, reciprocal matings were substituted if available. Otherwise, animals were substituted at random from within the line. This was rarely necessary.

Mensuration

In each of the series of measurements to be described, the slides or photographs were arranged by a third party in a random order with their identities hidden. Further details and discussion of this aspect of the design are given by Woolley (1969).

(i) *Midpiece length (nigrosin–eosin smears)*

The preparation of the nigrosin–eosin smears followed exactly the description given by Beatty & Sharma (1960). Since the selection experiment involved the study of small differences in size, measured over a period of more than 2 years, great attention was paid to standardizing the method of preparing the smears.

A description of the projection microscope used for this work has already been published (Woolley & Beatty, 1967). The system gave a linear magnification of 6136—a figure dependent upon a particular stage micrometer; the element of 'empty magnification' was required to avoid the use of micromethods in measuring the images. The constancy of the magnification was checked prior to the examination of the slides of each generation (except in the earlier generations) by repeatedly measuring the distance between a pair of easily identifiable points on a particular stage micrometer. These checks occasionally revealed significant differences but there was no overall trend and the data have not been adjusted. The inherent difficulty in verifying the constancy of such a measurement is that the observer's appreciation of a point or line may be slightly different on different occasions.

Ten spermatozoa were chosen per slide and for each the length of the midpiece, defined to include the neck region, was drawn on paper as a single pencil line. The spermatozoa drawn were chosen at random except for the following provisions: that the head was present, and that there was obviously a main piece (in this way broken midpieces were excluded); that the head was not grossly malformed; and that the insertion of the neck and the distal termination of the midpiece were clear and unobstructed. The drawings were measured with a rotameter read to $\frac{1}{32}$ in., which was itself calibrated against a steel rule. A slight change in the instrument, presumably due to wear, was noticed in the early stages of the experiment, and the data were adjusted accordingly.

(ii) *Midpiece length (verification in living spermatozoa)*

The sperm from the males of generation eleven was divided into two fractions: a nigrosin–eosin smear was made from half the spermatozoa by the method referred to; the rest were immobilised in a gelatine solution (8 % (w/v) in 0·85 % saline), and examined alive in a different microscope system.

This microscope was a Wild fitted with a camera lucida (Zeichentubus) and positive phase contrast optics. Using a 100× objective, and elevating the microscope above the drawing table, a magnification of 1559 was achieved. A heat filter was used. The lengths of ten midpieces were drawn as single pencil lines from each slide, and were measured at a later date with the rotameter. Again, the spermatozoa

were chosen at random except for the exclusion of those in which the midpiece was not lying more-or-less in one plane, and those with obvious morphological defects. At intervals during this work, a gelatine preparation was liquefied by warming after the drawings had been made. In all cases the motility of the spermatozoa was restored. Frequently, in fact, the 'immobilized' spermatozoa were seen to achieve slight vibratory motion against the resistance of the gelatine.

(iii) *Other dimensions*

In generation ten the total tail length and the midpiece length were measured in the same spermatozoa. The length of the tail, however, is approximately five times that of the midpiece, and, at the magnification of 6136, its image was inconveniently large. It was found that the necessary reduction in magnification could most simply be achieved by the unorthodox measure of removing the ocular; this produced an inverted, distortion-free image at a magnification of 1812. The length of the tail, from the insertion of the neck to the termination of the end-piece was recorded as a single pencil line and measured later with the rotameter. The choice of spermatozoa for this measurement had to be more critical on account of the frequent confusion between the tails of different cells. A measure of the head length (as defined by Braden, 1959) was also obtained for these same spermatozoa. Measurements were made with vernier calipers read to 0·1 mm. In the males of generation eleven, after drawing the midpiece length, the outline of the midpiece was drawn for each of the ten spermatozoa. This area was measured with a 'Haf' planimeter read to 0·05 cm^2. The mean width of the midpiece was obtained by dividing the projected area by the length.

(iv) *Electron microscopy*

Towards the end of the selection experiment, the three lines were examined in a search for differences in the ultrastructure of the spermatozoa. The development of the method used for this work, single-stage carbon replication, and the details of the procedure, have been described elsewhere (Woolley, 1970).

There were no *gross* differences in midpiece structure between the selected lines; indeed, none was expected. Attention was directed, therefore, to quantitative differences in structure, in particular, to the number of gyres (or turns or windings) made by the mitochondria in their helical arrangement around the flagellum. Because of the labour involved, the control line was not sampled as fully as the selected lines. Furthermore, the sampling of spermatozoa within males was also unbalanced: for technical reasons, some preparations were superior to others and more cells showing the required detail could be found and photographed. Most of the electron micrographs were made at a magnification of 4–5000 (actual).

In fact, there are predominantly two helices of mitochondria in the midpiece of the mouse spermatozoon (Challice, 1953; Woolley, 1970), and the 'number of gyres' is defined here to mean the sum of the gyres of the individual helices. The terminal annulus (Jensen's ring) was not counted as a gyre. The electron micrographs were

scored for the 'number of gyres' at the end of the investigation, each count being duplicated.

For comparative purposes, a nigrosin–eosin smear was also made from a sample of the contents of the vasa deferentia of each male and checked for the presence of spermatozoa before the remaining material was processed for electron microscopy. These slides were stored until the end of the series, when the midpiece length was drawn for 20 randomly chosen spermatozoa per slide in the way already described.

3. RESULTS

The change in the length of the midpiece under selection is shown in Fig. 1*a*, where the means of the family means have been plotted for each generation. It is clear from this that there has been a divergence between the two selected lines. After 13 generations of selection, this divergence amounted to 5·4 phenotypic standard deviations (or about 1μ) meaning that the high and low populations of male-means were virtually completely separate. Because the control line has shown no general tendency to move up or down, it seems that the asymmetry of the response is real. In fact, when the response is considered as deviations from the control line and plotted against the cumulative selection differential (Fig. 1*b*), the realized heritability in the low line ($0{\cdot}90 \pm 0{\cdot}04$) is significantly greater than that in the high line ($0{\cdot}61 \pm 0{\cdot}06$). A single value for the heritability, obtained from the linear regression of the divergence on the cumulative combined selection differentials (Fig. 1*c*) was $0{\cdot}76 \pm 0{\cdot}02$. These estimates of realized heritability are based upon the mean midpiece length of ten spermatozoa; they are expressed as h^2_{overall}, having been converted from $h^2_{\text{within-families}}$ by the method given by Falconer (1964). Thus

$$h^2_{\text{within-families}} = h^2_{\text{overall}}(1-r)/(1-t),$$

where r is the coefficient of relationship and t the intra-class correlation. For full sibs $r = 0{\cdot}5$ and $t = \sigma^2_F/[\sigma^2_F + \sigma^2_{M(F)}]$. The components of variance between and within families are given in Table 1. From an inspection of these data for each generation, no trends were detected, and therefore only the pooled estimates are presented. The value of $t_{\text{full sibs}}$, pooled over lines and generations, was $0{\cdot}350 \pm 0{\cdot}046$, the standard error having been calculated by an approximate method given by Becker (1967) and kindly explained to me by Dr W. G. Hill. The correlation between full sibs also estimates the heritability, specifying its upper limit: $t \geqslant \frac{1}{2}h^2$. This is explained in terms of the variance components,

$$t = [\tfrac{1}{2}V_A + \tfrac{1}{4}V_D + V_{Ec}]/V_P,$$

where V_A = additive variance, V_D = dominance variance, V_P = phenotypic variance and V_{Ec} = variance due to common environment. Since $2t = 0{\cdot}70 \pm 0{\cdot}09$, and, from the response to selection, $h^2 = 0{\cdot}76 \pm 0{\cdot}02$, it is concluded that dominance and common environment have contributed negligibly or not at all to the covariance.

Regarding the fertility of the selected lines, the ease with which the selection differential was maintained itself indicates no major impairment. The mean litter size (of those females which gave birth) and the percentage of sterile matings are given for each generation in Fig. 2. Evidently, there has been no differentiation

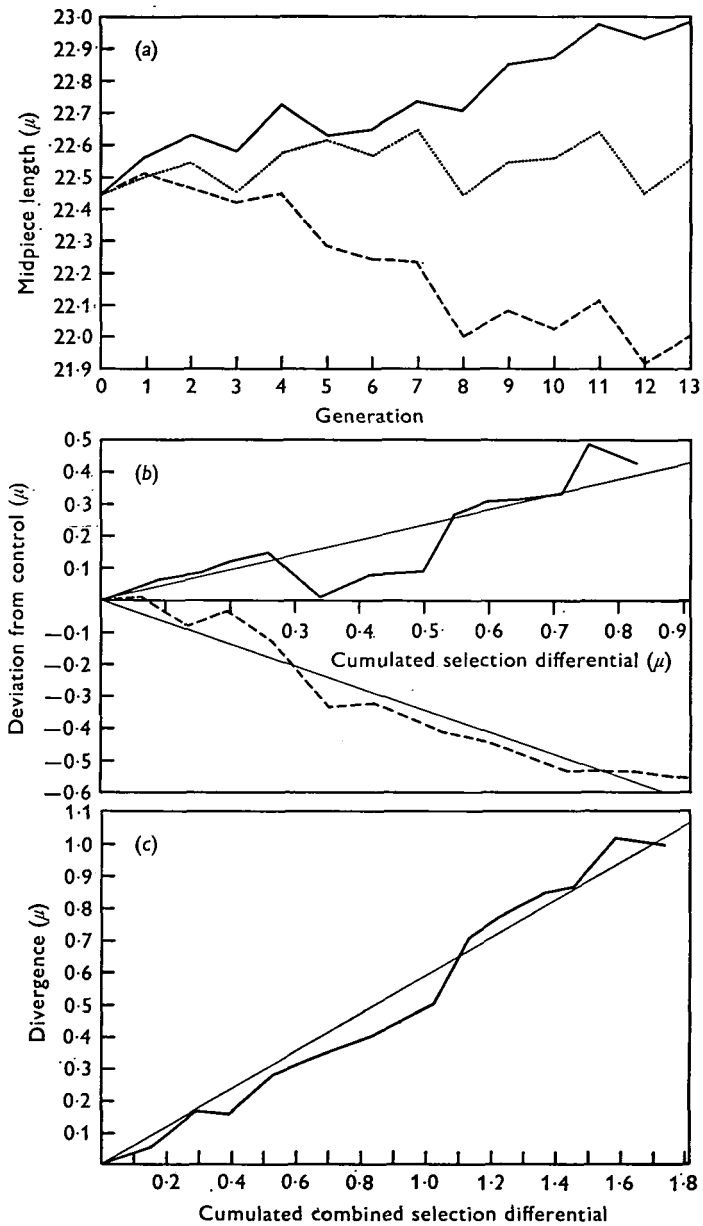

Fig. 1. (*a*) The response to selection. Each point is the mean of eight family means. (*b*) The response in the selected lines considered as deviations from the control line. The linear regressions have been made to pass through the origin. (*c*) The divergence between the high and low lines, with the linear regression fitted to pass through the origin. —— High line; ..... control line; – – – low line.

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between the lines in these characters. The overall percentage of sterile matings does show an upward trend, which is perhaps to be expected as the coefficient of inbreeding increases.

Table 1. *The variance components of midpiece length between families* (σ^2_F) *and between males, within families* ($\sigma^2_{M(F)}$), *with their degrees of freedom given in parentheses, and the phenotypic correlation between full sibs* (t). *Data in* μ^2

	σ^2_F	$\sigma^2_{M(F)}$	t
High line, generations 0–13 pooled	0·014 (90)	0·029 (275)	0·33
Control line, generations 0–13 pooled	0·023 (96)	0·038 (302)	0·38
Low line, generations 0–13 pooled	0·018 (89)	0·036 (276)	0·34
Pooled over lines and generations	0·019 (275)	0·035 (853)	0·35

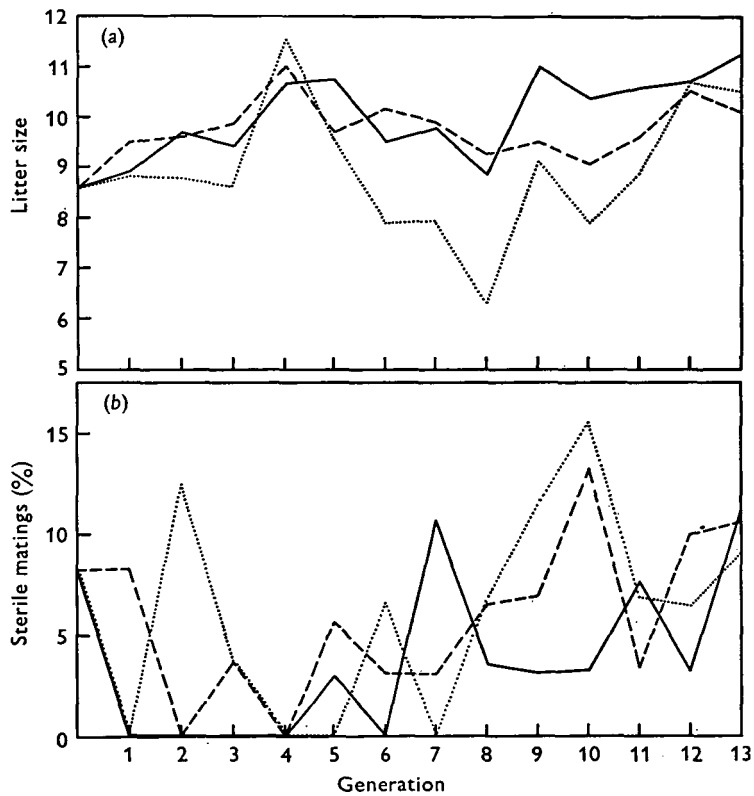

Fig. 2. (*a*) The litter size of those females which gave birth (means of family means). (*b*) The percentage of sterile matings. —— High line; ..... control line; – – – low line.

Confirmation that the midpiece length was really altered came from measurements on living spermatozoa from the males of generation 11. In Fig. 3 the mean length in the live cells is compared with the length in stained cells from the same males. The correlation coefficient was calculated within each line and then pooled to give $r = +0{\cdot}56 \pm 0{\cdot}09$ ($P < 0{\cdot}001$); and, from a t-test on the family means, the

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between-line differences in 'live' midpiece length were shown to be very highly significant ($P < 0{\cdot}001$). These results clearly verify that the change in length brought about by selection is real, and is independent of the method of smearing and nigrosin–eosin staining, etc.

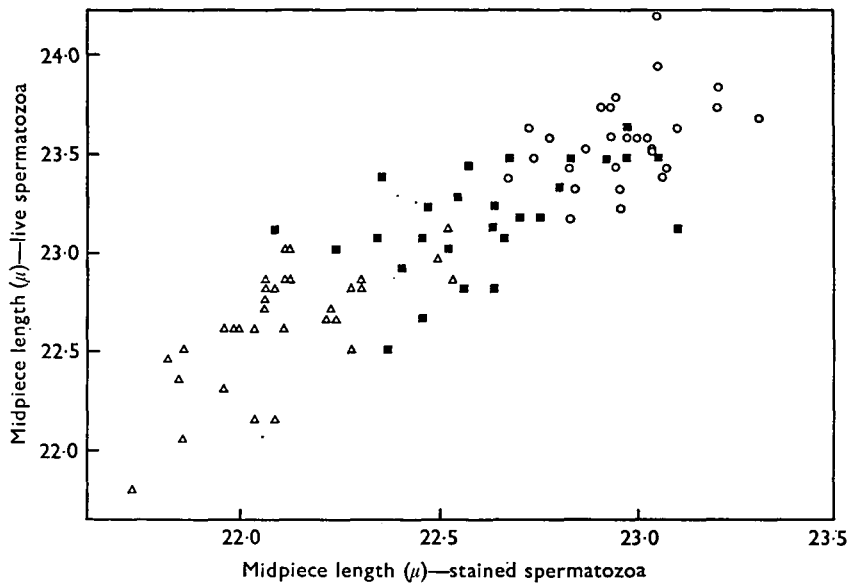

Fig. 3. A comparison of midpiece length in live and dead spermatozoa in generation 11. Each point is the mean for ten spermatozoa from one male. The magnification systems for the two measurements were different. ○, High line; ■, control line, △, low line.

Since Beatty & Sharma (1960) failed to demonstrate any between-strain variation in the projected area of the midpiece, it was conceivable that selection for a change in the length of the structure would be accompanied by an inverse change in its width. From the measurements made in generation 11, however, no such change in width can be detected. Instead, the projected area of the midpiece—the character actually measured—was significantly greater in the high line than in the low line. On calculating the mean midpiece width from the measurements of its area, no differences between the lines were apparent (Table 2 and Fig. 4).

It was of general interest to examine whether selection pressure on one part of the flagellum had had correlated effects on the growth of the entire flagellum, or indeed on the general cell size of the developing spermatids. To this end, measurements were made of the total length of the flagellum and the length of the spermatozoan head. Considering first the total length of the flagellum: from an analysis of variance (Table 2), no differences could be demonstrated between the three lines. In view of the variance of the measurements, however, it could not be concluded that the length of the remainder of the flagellum had changed in the opposite direction to the change in the midpiece. In fact, when the length of the 'main-piece + end-piece' was obtained by subtraction and analysed in the same manner,

the differences between the lines were again not significant. The relationship between the midpiece length and the length of the remainder of the flagellum is shown in Fig. 5; an incidental result is that, within the lines, there is a small positive correlation between the two lengths ($r = 0{\cdot}27 \pm 0{\cdot}10$, pooled within lines). It is thus not possible to decide, without a great many further measurements, whether the 'main-piece + end-piece' has remained unchanged in length during selection, or whether it has changed inversely and by the same amount as the change in the midpiece. It can be said, though, that selection for midpiece length has not been accompanied by a simply proportionate, i.e. approximately four-fold change in the length of the rest of the flagellum, as one might have expected if the selection were having a general effect on the growth of the spermatids.

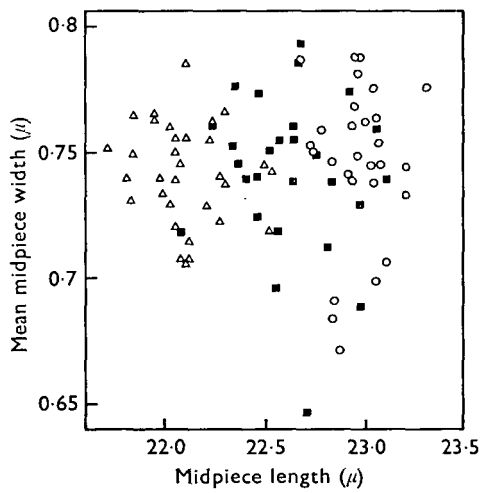

Fig. 4

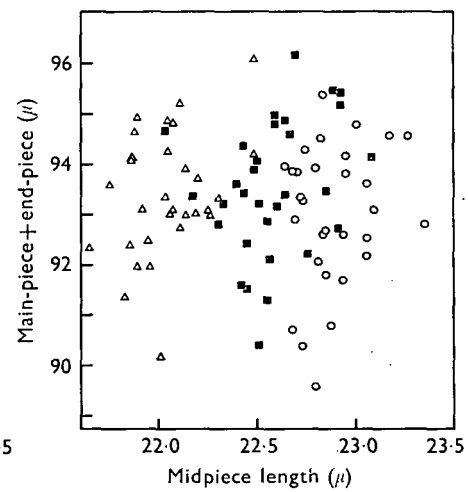

Fig. 5

Figs. 4 & 5. The relationships between midpiece length and midpiece width (generation 11); and between midpiece length and the length of the remainder of the flagellum (generation 10). Each point is the mean for ten spermatozoa from one male. ○, High line; ■, control line; △, low line.

Significant between-line variance was discovered in the length of the spermatozoan head (Table 2), but by applying a multiple range test it was found that only one difference was significant at the 0·05 level, namely, that the head length in the control line was greater than that in the high line (see Fig. 6). Whether this indicates a genetic correlation or merely a change due to drift has not been pursued at present.

The result of the study of the number of mitochondrial gyres constituting the midpiece is consistent with the observations on the length and width of the midpiece. An analysis of variance of the number of gyres revealed highly significant differences between the lines, between the males within the lines, and between the spermatozoa within the males (Table 3). However, the analysis should be interpreted cautiously because there was an irregularity in the sampling: the males

from the high and low lines were chosen from selected (i.e. more extreme) families. This was justified as an aid to estimating the regression coefficient of gyre number on midpiece length accurately; also, the technique was novel, and the accuracy

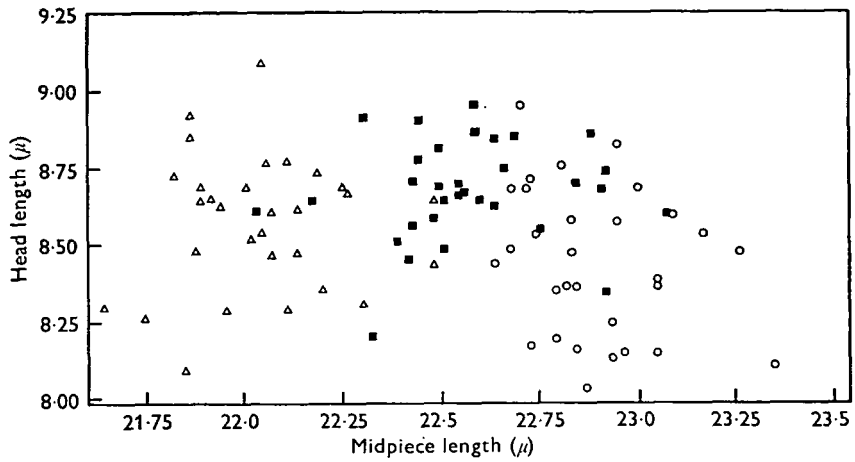

Fig. 6. Relationship between midpiece length and the length of the spermatozoan head in generation 10. Each point is the mean for 10 spermatozoa from one male. ○, High line; ■, control line; △, low line.

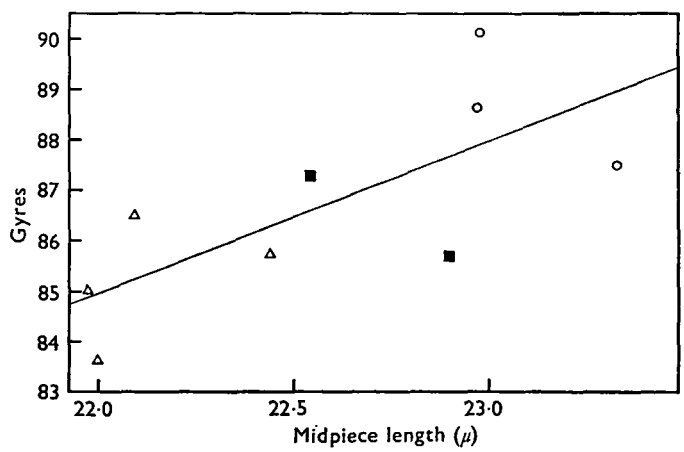

Fig. 7. Relationship between the mean midpiece length of 20 spermatozoa (as measured in the light microscope) and the mean number of mitochondrial gyres in a sample of spermatozoa from the same male. Each point represents one male. The linear regression has been weighted (see text). ○, High line; ■, control line; △, low line.

of the gyre counts could not be predicted. This fact may, however, have exaggerated the between-line variance. Nevertheless, despite this reservation, there were clearly significant differences between the lines as represented. In Fig. 7 the results are presented graphically. The linear regression was calculated after weighting the data to compensate for the different numbers of spermatozoa which were

used in estimating the mean gyre number of any male. The weighting procedure was taken from Falconer (1963). The regression coefficient of gyre number on midpiece length was $b = 3{\cdot}01 \pm 0{\cdot}64$ ($P = 0{\cdot}005 - 0{\cdot}001$). If the differences in midpiece length were accompanied by an exactly equivalent change in the number of mitochondrial gyres, the regression coefficient would be estimated thus, b = mean number of gyres/mean length of midpiece = 86·71/22·58 = 3·84. The difference

Table 2. *Three spermatozoan dimensions measured in either generation* 10* *or generation* 11†

Spermatozoan character	Mean of family means (μ)			Analyses of variance of family means				
	High line	Control line	Low line	Source	D.F.	M.S. (μ^2)	V.R.	P
Midpiece width†	0·763	0·761	0·764	Between lines	2	0·0000158	< 1	—
				Within lines	21	0·0007229		
Total length of tail*	116·03	116·08	115·55	Between lines	2	0·680	< 1	—
				Within lines	21	1·399		
Length of head*	8·475	8·676	8·575	Between lines	2	0·0805	4·14	0·05–0·025
				Within lines	21	0·0194		

Table 3. *Analysis of variance of mitochondrial gyre number*

Source	D.F.	M.S.	V.R.	P
Lines	2	383·54	12·87	0·01–0·005
Males (lines)	6	29·80	2·97	0·01–0·005
Sperm. (males)	127	10·03	5·31	< 0·005
Duplicate observations	136	1·89	—	—

between this and the observed coefficient was tested and found to be not significant ($P = 0{\cdot}4–0{\cdot}2$). It seems probable, then, that the number of mitochondrial gyres is an exact reflexion of the measured length of the midpiece. Using the measured regression coefficient $b = 3{\cdot}01$ the divergence between the selected lines in generation 13 is 2·9 mitochondrial gyres; using $b = 3{\cdot}84$, the difference becomes 3·8 gyres.

4. DISCUSSION

The realized heritability of the character 'midpiece length', $0{\cdot}76 \pm 0{\cdot}02$ is consistent with the approximate estimate of h^2 obtained previously from a son–sire regression (Woolley & Beatty, 1967) and is actually very similar to Napier's estimates of h^2 for the head length and breadth of rabbit spermatozoa ($0{\cdot}72 \pm 0{\cdot}18$ and $0{\cdot}71 \pm 0{\cdot}13$ respectively). Napier's results were also based on a sample of ten spermatozoa from each male. Now, because of the variation between the sperma-

tozoa of a male, the value obtained for h^2 will depend upon the number of spermatozoa sampled, and it is of interest to calculate how much higher the value would be if an infinite number of cells had been measured from each male. For this one needs an estimate of the within-male variance, which was, in fact, calculated only at the beginning and towards the end of the selection programme. The two values obtained, $0{\cdot}087\mu^2$ for generation 0 and $0{\cdot}081\mu^2$ for generation 11, were not, incidentally, significantly different. Taking account of this within-male variance $\sigma^2_{S(M)}$, the total phenotypic variance (σ^2_P) is now $\sigma^2_F + \sigma^2_{M(F)}$ (from Table 1) $+ \sigma^2_{S(M)/n}$, where $n = 10$, the number of spermatozoa sampled. Thus, if $0{\cdot}081\mu^2$ is used as the estimate of $\sigma^2_{S(M)}$, $\sigma^2_P = 0{\cdot}0611\mu^2$. The heritability for an infinitely large sample is then calculated,

$$h^2_\infty = \frac{\sigma^2_A}{\sigma^2_F + \sigma^2_{M(F)} + (\sigma^2_{S(M)/\infty})} = 0{\cdot}88.$$

This calculation further emphasizes the very high degree of genetic determination in the dimensions of spermatozoa. Whether the phenotype of other cells is similarly controlled is doubtful, though there have been few studies. It seems likely that only those cells which are similarly limited in function—such as erythrocytes (Kashiwabara, 1964)—will show a high degree of genetic determination. One might expect the variability in the characteristics of functionally more complex cells to be largely due to environmental factors.

The projected area of the midpiece, unlike the length, had not been found to vary significantly between eight inbred strains of mice (Beatty & Sharma, 1960), indicating, perhaps, a constancy of mitochondrial material. In the present work, however, an increase or decrease in the length of the midpiece has been accompanied by a proportionate change in its projected area. The width of the structure has remained the same. Therefore, assuming that the midpiece has retained its circular cross-section during the experiment, it follows that the change in length must have involved a change in the volume of the midpiece. This conclusion has been re-inforced by the finding that the number of mitochondrial gyres has increased or decreased in the two selected lines. While it can be said, then, that the volume of mitochondrial material in the spermatozoa has been changed, it has not been proved that the number of the component mitochondria has changed, since the same results might be seen if each individual mitochondrion had changed its length and remained of the same width. To establish the most likely change in the development of the spermatozoon, we should consider how the distinction between the main-piece and midpiece arises in the course of spermatogenesis. According to Nicander (1962), the fibrous sheeth which encloses the main-piece of the flagellum is laid down in the late cap phase of spermateleosis; this has recently been confirmed from a study of carbon replicas of spermatids (Woolley, 1970). Developmentally, this means that the distinction between main-piece and midpiece is present in the flagellum before the migration and apposition of the mitochondria. The length of the midpiece may be thought of as determined by the extent of the deposition of the fibrous sheath protein—its absence from the

proximal part of the flagellum establishing the 'midpiece length'. The simplest interpretation of the effects of this selection experiment is that it has changed the frequencies of those genes which in some way control the pattern of protein deposition on the flagellum, thus altering the length of the axis to which the mitochondria may be attracted. In consequence, the number of mitochondria which can be accommodated in a single layer along this axis has altered. There is much evidence to show that there are many more mitochondria in the spermatid cytoplasm than are incorporated into the developing midpiece (e.g. Dietert, 1966).

The final, pertinent question to be raised is what effect the difference in mitochondrial volume which has been achieved by selection might have on the functional capacity of the spermatozoon, in particular its ability to effect fertilization. The opportunity has been taken to examine this question experimentally, and the findings will be reported in a subsequent publication.

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