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SHORT PAPERS

A test of the functional significance of the quantity of mitochondria in the spermatozoa of mice

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SUMMARY

Following an experiment in which mice had been selected for the length of the mitochondrial section (midpiece) of their sperm tails, an attempt has been made to determine experimentally the adaptive significance of this character; this was by the artificial insemination of mixtures of selected and unselected (control) spermatozoa, and the subsequent disclosure of their competitive fertilizing ability in the paternity of the offspring. After the birth of nearly 500 offspring, there was no indication that the control cells were—by this criterion—functionally superior. From the discrepancy between the pairs of males sampled, however, it is possible that other biological or technical factors have been important.

1. INTRODUCTION

The spermatozoa of different species are highly diverse morphologically, yet within a given species the variability between the individual males is unusually low (Beatty, 1970). In discussing the specificity of sperm structure, Friend (1936) took the view that the obvious differences in nucleus shape between rodent species are probably non-adaptive, conferring no advantage to the animals which bear them. It is quite common, on the other hand, for students of comparative sperm structure to suppose that structural specificity will coincide with functional adaptiveness (e.g. Fawcett (1958) referring to the length of the mitochondrial part of the flagellum). Rothschild (1962) has also noticed how, in speculating on the 'meaning' of structure in spermatozoa, different authorities adopt opposing positions. The most important relevant fact is that the dimensions of spermatozoa in mammals have rather high heritabilities (Beatty, 1970); and, generally, it is characters of low heritability which have an unmistakable relationship with fitness (Falconer, 1960).

In the present work an experimental test of adaptiveness has been attempted, using mice which had been artificially selected over 13 generations for the length of the spermatozoan midpiece—in effect, mice selected for the quantity of mitochondria in their spermatozoa (Woolley, 1970). The aim has been to inseminate numerically balanced mixtures of selected and unselected spermatozoa, thus allowing them to compete in the female tract. The 'fitness' of the cells—their efficiency in achieving fertilization—would be apparent from the paternity of the offspring. The success of an individual's spermatozoa in this type of experiment ('heterospermic' insemination) does have a genetic basis in the mouse (Edwards, 1955), and in other species has been found to be an indicator of the individual's homospermic fertility (Beatty, 1960; Beatty *et al.* 1969).

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2. EXPERIMENTAL PROCEDURES

Mice were available from the lines selected for long and short spermatozoan midpiece (HIGH and LOW lines respectively), and from the unselected line (CONTROL). The triple semen mixture HIGH + CONTROL + LOW, the most powerful statistically, was not attempted for technical reasons; of the three double mixtures, only two could be attempted with sufficient thoroughness and so the HIGH + LOW mixture was omitted as being least relevant. There were thus two experimental series of sperm mixtures: a comparison of the long midpiece line with the control (series 1), and a comparison of the short midpiece line with the control (series 2). Each series consisted of a number of experiments, each one being the comparison of two males, one from each line. Each sperm mixture was divided up and inseminated into eight females. Some common coat colour genes which were segregating in the lines (*a*, *c*, *b*) were used to mark the paternity of the offspring. Testing of the males' genotypes was usually necessary beforehand and males which did not require testing were allowed similar sexual experience. Thus all the males used were known to be fertile in natural matings. All the inseminations were done in JU inbred females of genetic constitution *cc*, *aa*.

Ovulation and oestrus were induced in virgin females by a gonadotrophin régime (Land & Falconer, 1969) such that ovulation was expected at 01.00 h. Sperm was taken from the vasa deferentia and distal segment of the caudae epididymides of the two males 30 min *post mortem*. A small fraction from each was used to make a stained smear and the remainder of each sample was suspended in a phosphate-buffered saline (pH 7·2) for 20 min to allow dispersion. Then haemacytometer counts were made and appropriate volumes were taken, giving approximately equal numbers of spermatozoa. After thorough mixing, the final suspension was diluted with a few drops of TC 199 (McGaughey, Marston & Chang, 1968). Following the findings of these same authors, the females were inseminated 3 h before ovulation at 22.00 h. About 0·07 ml of suspension ($1–4 \times 10^6$ spermatozoa) was introduced through the cervix (method of Dziuk & Runner, 1960) with the females lightly etherized. Immediately after insemination, each female was paired with a vasectomized male of proven sterility and examined next morning for a plug. Those females not plugged were given 5 mg (s.c.) of 'Depo-Provera' (Upjohn Co.) in an attempt to maintain pregnancy (P. S. Grant, 1969, personal communication). Offspring were scored either at 18 days gestation or 5 days after birth, depending on the genotypes expected. This established the 'paternity ratio' for each litter. Females whose pregnancies were maintained by 'Depo-Provera' were killed on day 20 and the neonates fostered. A general discussion of the artificial insemination of mice is given by Wolfe (1967).

3. RESULTS

In no experiment was there significant heterogeneity between the paternity ratios of the individual litters, indicating that the inseminates were homogeneous. Also, treatment with 'Depo-Provera' had no effect on the paternity ratio (though it did reduce the litter size significantly and was less effective than plugging in maintaining pregnancy). Therefore, because there was no discrepancy within experiments, the pooled data from each experiment are given (Table 1). The most striking result is the very considerable heterogeneity between the experiments of each series. This prohibits serious consideration of the overall ratios for the series. For analysis, the ratios have been expressed as proportions, but since there appear to be real differences between experiments these proportions cannot simply be weighted according to the numbers of offspring in each experiment. A very crude but nevertheless unbiased analysis has therefore been performed: in each series, the mean proportion of offspring sired by males from one line has been calculated, together with the standard error of the mean. This calculation, which

gives equal weight to each experiment, indicates that in neither series does the mean proportion differ significantly from the expectation of 0·5. In fact, in both series the tendency has been for the control line to be *less* successful than the selected lines in achieving fertilization; clearly then, there is no indication in these results that control spermatozoa, carrying the 'normal' quantity of mitochondria, are competitively superior to those which have been changed by artificial selection.

Table 1. *The results of the heterospermic inseminations, pooled over litters*

Series I. CONTROL versus HIGH

Expt no.	No. of litters	No. of offspring scored	Paternity		Proportion High
			Control	High	
3	8	32	0	32	1·00
4	6	25	17	8	0·32
5	6	30	5	25	0·83
9	7	45	25	20	0·44
10	8	22	0	22	1·00
14	6	73	14	59	0·81
16	6	51	35	16	0·31
	Totals	278	96	182	
			Mean ± S.E.		0·67 ± 0·12

Series 2. CONTROL versus LOW

Expt no.	No. of litters	No. of offspring scored	Paternity		Proportion Low
			Control	Low	
1	2	9	2	7	0·78
2	4	40	11	29	0·73
6	6	41	0	41	1·00
8	8	49	14	35	0·71
11	7	45	32	13	0·29
12	5	11	4	7	0·64
13	3	1	1	0	—
15	2	1	0	1	—
	Totals	197	64	133	
			Mean ± S.E.		0·69 ± 0·09

Measurements made retrospectively from stained smears have confirmed that the males used in these comparisons were indeed different in the mean midpiece length of their spermatozoa. The mean deviations of the selected males from control were $+0{\cdot}44 \pm 0{\cdot}14\ \mu$ (series 1) and $-0{\cdot}64 \pm 0{\cdot}10\ \mu$ (series 2).

4. DISCUSSION

In comparing the heterospermic performance of mice, Edwards (1955) found there was reasonable consistency between the pairs of males sampled from two inbred lines; in the present inseminations, using outbred strains, there has been no such consistency. The lack of genetic uniformity, then, is a possible explanation. It is also possible, however, that the discrepancy between experiments has a technical rather than a biological

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origin. At this stage, specific technical factors have not been incriminated (one factor—the order of preparing the sperm suspensions—has actually been ruled out), but the occasional failure of an inseminate to produce the expected number of offspring does indicate the need for more basic research on the procedure for A.I. in mice. Nevertheless, great effort has been made to standardize the handling of the animals and their spermatozoa, and a provisional interpretation of the results—that the findings have a biological foundation, probably reflecting the genetic diversity within the lines, and that they reveal no overall differences between the lines—is suggested. This leads to the conclusion that the midpiece length in the control spermatozoa is not at a *functional* optimum (in so far as heterospermic success is a test of total function). This tentative conclusion, drawn from experimental evidence, has the merit of being consistent with the expectation made, largely on theoretical grounds, from the high heritability. The length of the midpiece, and, one suspects, other aspects of spermatozoan morphology, would seem to belong to that somewhat enigmatic group of characters which combine low phenotypic variability with apparent selective neutrality.

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