Selective fertilization at the T-locus of the mouse

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1. INTRODUCTION

The route to fertilization is through gametogenesis, transport of gametes to the site of fertilization, and conjugation. Fertilization is expected to lead to equal recovery in the newly-formed zygotes of alleles that were heterozygous in the parents. The term selective fertilization has been applied to many instances when the recovery was unequal, but it is perhaps not generally realized from what devious sources and with what individually indentifiable genetic consequences the inequality may arise. Accordingly, an analysis of selective fertilization in all its possible forms is sketched in the preliminary part of this paper.

However, the main reason for the paper is to describe a new form of selective fertilization from the mouse. It will be shown that spermatozoa transmitting the t^e allele of the T-locus did not fertilize equal numbers of alternative eggs from heterozygous females. The T-locus, which gets its symbol from effects on tail length, has been extensively reviewed by Grüneberg (1952) and by Dunn & Gluecksohn-Waelsch (1953). It has many alleles, but only the three whose zygotes are set out in Table 1 need be considered for present purposes. The alleles T, $+$, and t^e are

Table 1. *List of genotypes at the T-locus*

Symbol	Full name
$+$ $+$	Normal-tailed, normal homozygote
$+$ t^e	Normal-tailed, carrier
t^e t^e	Tailless-Edinburgh homozygote (dies *in utero*)
T $+$	Short-tailed
T t^e	Tailless
T T	Brachyury homozygote (dies *in utero*)

brachyury, normal and tailless-Edinburgh, respectively. Both t^e and T are lethal to homozygotes, death occurring *in utero*. Other features of t^e are that it is one of many t genes (mostly lethals) which are all without phenotypic effects in heterozygous compounds with the normal gene, but which give tailless zygotes with brachury; and, like most other t genes, t^e is transmitted by heterozygous males to the great majority of their effective spermatozoa. Dunn (1953) has anticipated that t genes with high degrees of transmission in the spermatozoa would be widespread among wild mice, and this is indeed so. The exact mathematical relation between the frequency of heterozygotes and the frequency with which heterozygous

males transmit t to their spermatozoa has been worked out by Bruck (1957), but neither natural populations (Dunn, Beasley & Tinker, 1958) nor the only laboratory population which has been studied (Bateman, 1960) agrees with Bruck's predictions. As yet, no satisfactory explanation of these discrepancies has been found, but the possibility that eggs and spermatozoa might have conjugated selectively had not been investigated. However, it has been shown in the laboratory population (Bateman, 1960) that in matings with $+t^e$ males, fertilization of T eggs was less frequently due to t^e spermatozoa (87%) than were fertilizations of $+$ and t^e eggs (96–100%). As the comparison cut across females of different genotypes it was not possible to determine from the data whether any part of the difference resided with the eggs themselves. However, new evidence will be presented here that the alternative genotypes of eggs found in heterozygous females do modify the advantages in fertilization that t^e spermatozoa have over those carrying the $+$ or T alleles.

2. TYPES OF FERTILIZATION

Selective fertilization may be defined as unequal sampling in newly formed zygotes of genes that were heterozygous in parents. The selection may occur during gametogenesis, transport or conjugation. Figure 1 depicts the various consequences in terms of mothers heterozygous for alleles a and b, and fathers heterozygous for c and d. The ratio of the four zygotic classes ac, ad, bc, bd is represented by the relative areas of the respective cells whose dimensions are determined by the proportions in which parental genes are combined by the uniting gametes. Bold divisions demarcate classes that are expected to be of equal size.
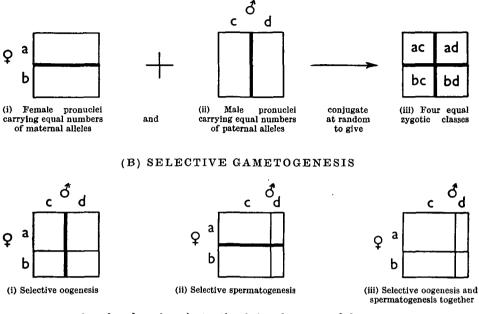
Normal fertilization (Fig. 1A)

In normal matings between heterozygotes, equal numbers of maternal alleles (represented by two equal rectangles one above the other, Fig. 1A i) combine with equal numbers of paternal alleles (two equal rectangles side by side, Fig. 1A ii) to give four zygotic classes of equal size (Fig. 1A iii). From this basic Mendelian ratio of 1 : 1 : 1 : 1 the more familiar 3 : 1, 1 : 2 : 1 and 1 : 1 ratios are derived to accord with the number of alleles and their dominance relations. Mendelian ratios may be disturbed *after* the formation of zygotes by phenotypic overlapping and different zygotic viabilities. As the aberrant segregations of most 'bad' genes are accommodated in these terms, proof of selective fertilization can be accepted only after these sources of non-Mendelian segregation are rigorously excluded.
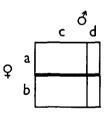
Selective gametogenesis (Fig. 1B)

If certain alleles tend to be eliminated from the germ line by the meiotic process, this is selective gametogenesis. The resulting inequality of parental alleles in the offspring is of course uninfluenced by conditions of mating. Thus the predominantly male or female progenies of mice in lines bred for high or low blood pH are almost certainly the product of selective spermatogenesis. The father's origin determines the predominant sex of the offspring in outcrosses even when insemination is
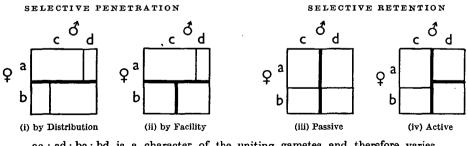
(A) NORMAL FERTILIZATION

(i) Female pronuclei carrying equal numbers of maternal alleles

and

(ii) Male pronuclei carrying equal numbers of paternal alleles

conjugate at random to give

(iii) Four equal zygotic classes

(B) SELECTIVE GAMETOGENESIS

(i) Selective oogenesis

(ii) Selective spermatogenesis

(iii) Selective oogenesis and spermatogenesis together

a : b and c : d are invariant ratios, being characters of the parents.

(C) SELECTIVE SPERM TRANSPORT

c : d is a character of the male or female genital tracts and may vary with the mother and with appropriate techniques of semen collection and insemination.

(D) SELECTIVE CONJUGATION

SELECTIVE PENETRATION

SELECTIVE RETENTION

(i) by Distribution

(ii) by Facility

(iii) Passive

(iv) Active

ac : ad : bc : bd is a character of the uniting gametes and therefore varies according to the genotypes of both parents.

Fig. 1. Causes of selective fertilization, and results expected.

artificial and semen is taken from the vas deferens (Weir, 1958). Likewise in male *Drosophila* that are heterozygous for segregation distorter. Close synapsis in spermatogenesis of normal and SD homologues is thought to break the normal chromosome with the result that normal, viable spermatozoa are rarely produced and SD is recovered in 90% of the offspring (Sandler, Hiraizumi & Sandler, 1959). Fully authenticated cases of selective gametogenesis are the expulsion of larger chromosomes of unequal pairs in polar bodies (Sandler & Novitski, 1957); replication of X chromosomes with compensatory loss of Y's, as cited by Wallace (1948) for sex-ratio strains of *Drosophila*; and gene conversion (Lindegren & Lindegren, 1956).

Selective transport (Fig. 1c)

Selective transport of gametes is indicated when changing the conditions of mating or changing the mate affects gene frequencies in the offspring. Vertebrate eggs are not open to such selection because gametogenesis and conjugation overlap. On the other hand, vertebrate spermatozoa may be selected during transport in both the father's and mother's genital tracts. Thus, in contradistinction to selective spermatogenesis (Fig. 1b ii), the anomalies of selective sperm transport can vary with the mother, and according to such conditions as (i) whether matings are natural or artificial, (ii) where the spermatozoa are taken from and where inseminated, (iii) the time spermatozoa spend in the tracts. Such anomalies have been described for mice (Braden, 1958), where the timing of coitus in respect of oestrus alters the relative fertilizing capacities of spermatozoa carrying *t* genes and their alleles. According to Braden & Gluecksohn-Waelsch (1958) the junction of the uterus with the Fallopian tubes is a site of stringent sperm selection. In many monoecious plants self-fertilization is inhibited by the slow growth of pollen tubes transporting genes that are the same as ones present in the style.

Selective conjugation (Fig. 1d)

In matings between heterozygotes it may be possible to show that nuclear conjugations are not randomly disposed among gametes which reach the site of fertilization. Selective conjugation reflects special associations among gametic nuclei carrying particular alleles. Eggs of non-vertebrate organisms undergo both meiotic divisions before becoming available for spermatozoa to penetrate. The eggs of these organisms are therefore fully differentiated at penetration in regard to the genes they carry, so that in these organisms selective conjugation of nuclei is simply the consequence of 'selective penetration' among gametes (Fig. 1d i–ii).

The significance of this point is clarified when it is remembered that vertebrate eggs are penetrated after completing only their first meiotic division (Beatty, 1957). Although this division is fully reductional in the sense of halving the number of bivalent chromosomes and of segregating alternative alleles in non-crossover segments, heterozygosity in crossover segments remains until sister chromatids separate at the second division (White, 1945). Non-crossover segments are likely to include loci that are close to the centromeres; and crossover segments the more distal loci. Vertebrate eggs can therefore be categorized in regard to a locus for

which conjugation is selective according to whether this locus segregates at the first or second meiotic division. Eggs of the first category are distinguished before penetration by the one allele each carries, and like non-vertebrate eggs are therefore accessible to selective penetration. This is clearly ruled out if the locus does not segregate till the second meiotic division, because in this case the eggs had uniformly carried both alleles when they were penetrated; but when such eggs divide again, should the spermatozoa retain selected alleles and eliminate the alternatives in the second polar bodies, selective conjugation of a different type will be effected. This type is distinguished as 'selective retention' (Fig. 1D iii–iv). The distinction is significant genetically, as the mode of conjugation determines which parent's alleles will be unequally inherited. Assuming all eggs are fertilized, the mechanics of normal meiosis in selective penetration ensures equal recovery of the mother's genes. However, the superior ability to fertilize certain eggs which is given to spermatozoa carrying one of the father's alleles biases the recovery of these. (As described later in this paper, these features appear in fertilizations involving t^e spermatozoa and mark the anomalies there as being due to selective penetration.) The contrary features obtain for selective retention. Here, the uniformity of penetrated eggs ensures equal entry of the two classes of spermatozoa into the zygotes, while spermatic interference in the egg's second meiotic division results in unequal participation of the mother's genes. The existence of selective retention has not yet been established, although its prerequisites are perhaps not very different from selective oogenesis.

It is of value to subdivide both forms of selective conjugation. For example, selective penetration may be the result of inequalities in the distribution of spermatozoa about the eggs, or of the unequal facility with which individual spermatozoa penetrate them. In the mouse, where eggs are tightly clustered at the site of fertilization (Braden, private communication) and few spermatozoa reach them (Braden, 1957), it could happen that a surfeit of one type of spermatozoa attracted to some eggs sufficiently depletes these spermatozoa elsewhere to produce a reciprocal bias in their capacity to fertilize the alternative eggs (Fig. 1D i). On the other hand, when the spermatozoa are evenly distributed, the facility with which some spermatozoa penetrate half of the eggs would not affect the segregation of the father's genes in respect of the others (Fig. 1D ii). The passive and active varieties of selective retention are determined according as the gene for which the father is heterozygous confers the selective property on all his spermatozoa, so that both his alleles cause unequal recovery of the mother's genes (Fig. 1D iii), or only on that half to which the gene itself is transmitted, when the maternal genes are recovered normally in zygotes derived from the other spermatozoa (Fig. 1D iv). It is interesting to note that paternal influences on spermatozoa are considered by Braden & Gluecksohn-Waelsch (1958) to be responsible for the infertility of viable tt males.

The types of selective fertilization have been depicted separately merely as a descriptive convenience, and once alleles find phenotypic expression in gametes there seems no reason why selective gametogenesis, transport and conjugation should not concur. Indeed, the results presented in the later sections of this paper

show that besides the anomalies in the segregation of t^e that are attributed to selective transport, other anomalies are only accountable in terms of selective penetration.

3. MATERIAL

Mice used in the investigation into selective conjugation in respect of the T-locus were descended from a brachyury stock (B stock) and the laboratory stock (L stock) in which the t^e gene was discovered. The supply of Tt^e, $T+$ and $+t^e$ animals required by the investigation was obtained from matings between Tt^e mothers and $T+$ fathers. These, being F_1's or backcross hybrids to the L stock, were either one-half or three-quarters L stock, whereas the experimental animals were one-half or five-eighths L stock as at least one of their parents was an F_1. This material was the more homogeneous as the t^e and $+$ genes always derived from the L stock and only the T gene derived from B stock.

4. METHODS

It will be remembered from the earlier results reviewed in this Introduction that the lethal gene t^e was transmitted by heterozygous males in unusually high frequency to their offspring in general, but that this phenomenon alone (believed to be due to selective transport of t^e spermatozoa to the site of fertilization) could not entirely account for the frequency of lethal heterozygotes in the original population. It was thought that the discrepancy might be made good if t^e spermatozoa, after reaching the site of fertilization, then selected the eggs with which they conjugated. Reference to Fig. 1D i–iv shows that evidence on selective conjugation may be obtained when parents, both of which must be heterozygotes, are chosen in such a way that four zygotic classes are distinguishable in their offspring. In this experiment expected frequencies of the zygotes are written in terms of s (respecting zygotes derived from selective fertilizations by t^e spermatozoa) and r (from the residual fertilizations by spermatozoa carrying an alternative allele). Both s and r are given subscripts T, $+$ and t to designate the allele provided by the egg. This symbolism does not presume specific types of fertilization, but we shall use it solely to gain evidence on selective conjugation, as it is unnecessary to reduplicate the evidence on other types of fertilization at this locus. Figure 2 depicts the results expected from each form of selective conjugation for two types of mating actually made. The expectations are seen to be complicated both by the inequality of the two classes of spermatozoa reaching the site of fertilization, and by the inconstancy of their relative sizes between one mating and another (Smith, 1956; Braden, 1958). Further reference will be made to this figure when interpreting the results of these matings.

Data on selective conjugation could be obtained from three kinds of heterozygous females (Tt^e, $T+$ and $+t^e$) in matings to two kinds of males heterozygous for t^e (Tt^e and $+t^e$). However, out of the six possible types of mating only three (numbered types 4–6 in Tables 2–5) were made, as the others gave less than four distinguishable classes of zygote. Unfortunately, the death of either TT or t^et^e embryos in each

mating left only three recordable classes at birth. Though these were adequate for general tests of selective conjugation, its nature could not be determined from these matings alone. Consequently, special control matings (type 3) were made where,
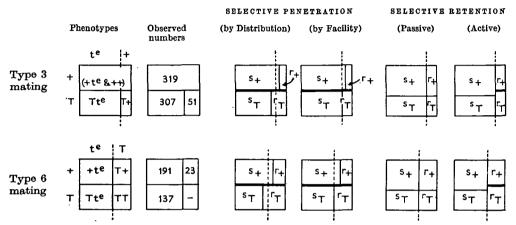

Fig. 2. Interaction of selective transport of t^e spermatozoa with the four forms of selective conjugation: consequences for offspring of $T+$ females mated with $+t^e$ and Tt^e males. The dotted vertical line represents the frequencies in which t^e and $+$ or T spermatozoa reach the site of fertilization.

although two of the products of conjugation were indistinguishable, all four products survived.

Finally, two other control matings were put up to give information on the extent of phenotypic overlapping and on the viability of Tt^e, $T+$ and $+t^e$ zygotes, for

Table 2. *Expectation of progeny in control and experimental families*

	Parents	Progeny		
Type	♀ × ♂	Tt^e :	$T+$:	$+$
	Controls			
1	$Tt^e \times T+$	1 :	1 :	1
2	$+t^e \times T+$	1 :	1 :	2
3	$T+ \times +t^e$	s_T :	r_T :	$s_+ : r_+$
		1		1
	Experimentals			
4	$Tt^e \times +t^e$	s_T :	r_T :	r_t
5	$+t^e \times Tt^e$	r_t :	r_+ :	s_+
6	$T+ \times Tt^e$	s_T :	r_+ :	s_+

without this information the frequencies of gametic conjugations in experimental matings could not be properly assessed. Control matings types 1 and 2, using Tt^e and $+t^e$ females respectively, were expected to yield their offspring in Mendelian ratios. It so happened that type 3 matings, using $T+$ females, provided no evidence

of selective retention and could therefore be regarded as controls, too. In this way, control and experimental matings used females of identical genotypes as regards the T-locus. Similarity of genetic background was assured by allocating both sisters and brothers equally to the two groups of matings. Expectations of progeny for the two groups are set out in Table 2.

Between six and twenty-five matings were made of each type. Post-natal inviability of Tt^e mice made it difficult to obtain many matings using such females. Offspring were scored on their day of birth as having zero, short or normal tails, and were discarded. The dams were then re-mated. As many litters were raised as were possible during the useful reproductive lives of the parents. Parents that gave rise to aberrant progenies were finally bred to other mates to ensure that no errors had been made in classifying parental genotypes.

5. RESULTS

Type 1 and 2 control families

Matings of types 1 and 2 were made as controls on viability and phenotypic over-lapping. Progeny totals and expectations are given in Table 3. The segregations are perfectly normal and are consistent among individual families (chi-squares for

Table 3. *Segregation in control families*

	Parents		Progeny				Expected ratio				χ^2	d.f.	P
Type	♀	× ♂	Tt^e	$T+$	$+$								
1	Tt^e	$\times T+$	74	74	72	1 :	1 :	1			0·04	2	0·98
2	$+t^e$	$\times T+$	199	175	388	1 :	1 :	2			1·77	2	0·45
3	$T+$	$\times +t^e$	307	51	319	s_T :	r_T :	1			2·25	1	0·15
				358	319		1	:	1				

heterogeneity were $\chi^2_8 = 1\cdot52$, $P = 0\cdot99$; and $\chi^2_{24} = 21\cdot96$, $P = 0\cdot60$). It is concluded that tailless, short-tailed and normal-tailed foetuses were neither unequal in viability nor subject to serious phenotypic overlapping. (Indeed, only one case of phenotypic overlapping occurred among 211 individuals whose genotypes were definitely known. This case was due to a genetically short-tailed mouse who had the appearance of a tailless one.) These conclusions strictly apply to offspring of tailless and normal-tailed females from whom the data were gathered. However, it would be fair to assume that the situation would be the same for the remaining short-tailed class of experimental females that are both phenotypically and genotypically inter-mediate. It is evident that the disturbed segregations among the progeny of $+t^e$ and Tt^e fathers are wholly consequent upon selective fertilization.

Type 3 control families

Type 3 control matings were made to determine whether irregular conjugations from heterozygous t^e males could be attributed to selective retention, as would have been indicated if maternal genes in this mating had segregated abnormally. But the

maternal genes are roughly equally represented (Table 3), and the majority of constituent families agree in this ($\chi_{10}^2 = 16\cdot62$; $P = 0\cdot09$).

However, this is not highly critical evidence that selective conjugation with t^e spermatozoa was by selective penetration, and it is desirable to extend the field from which evidence may be drawn. Accordingly we shall now attempt to compare segregation in matings of types 3 and 6 (Fig. 2), where different heterozygous t^e males (Tt^e and $+t^e$) were used on the same ($T+$) females. In the case of passive selective retention it is clearly meaningful, no matter how $s:r$ ratios differ between mating types, to compare the numbers of $+$ and T eggs fertilized by all spermatozoa in type 3 matings with the numbers fertilized only by t^e spermatozoa in type 6. The expectation is that $(s_+ + r_+)/(s_T + r_T)$ for type 3 $= s_+/s_T$ for type 6. There is practically the same expectation from active selective retention considering 86% eggs were fertilized by t^e spermatozoa. On the other hand, in the case of selective penetration there is neither reason why the two sides of the above equation should have similar values, nor any alternative meaningful comparison to be made. Consequently, contingency of the two sets of figures (319 : 358 for type 3, and 191 : 137 for type 6) would be evidence of selective retention. However, $\chi_1^2 = 10\cdot91$, $P = 0\cdot001$; and the obvious lack of contingency firmly supports the previous evidence of selective penetration. It is inferred that wherever selective conjugation is encountered in the experimental matings it will be the result of selective penetration. Arising from this conclusion is the practical point for interpreting the experimental findings: the larger of two r-values from a family implies a smaller value of the corresponding s. This reciprocal relation does not hold for selective retention.

Experimental families

Data on the progeny totals for the experimental families are given in Table 4. In each there is an expectation that two of the progeny classes will occur approximately equally (both having the expectation s, or both r) whose equality over all families of the same type can be statistically tested. The results of these tests are given in Table 4. Uniformity tests were also made to determine whether the pairs of s-values or the pairs of r-values bore the same relation to each other within each family as in the type as a whole. These yielded the following χ^2's: $\chi_5^2 = 3\cdot64$, $P = 0\cdot60$ (type 4); $\chi_{11}^2 = 22\cdot17$, $P = 0\cdot02$ (type 5); $\chi_8^2 = 9\cdot30$, $P = 0\cdot30$ (type 6). Families of type 5 were the only ones to conform to expectation as a group, but were also the only ones among which there was significant heterogeneity. Thus, in some matings of this type, and in general for matings of the other two types, the number of eggs fertilized by t^e

Table 4. *Segregation in experimental families*

Type	Parents ♀ × ♂	Progeny Tt^e	$T+$	$+$	Expected ratio	Equality of s- or r-values Comparison	χ_1^2	P
4	$Tt^e \times +t^e$	164	23	43	$s_T : r_T : r_t$	$r_T : r_t$	6·06	0·013
5	$+t^e \times Tt^e$	67	54	155	$r_t : r_+ : s_+$	$r_t : r_+$	1·40	0·24
6	$T+ \times Tt^e$	137	23	191	$s_T : r_+ : s_+$	$s_T : s_+$	8·89	0·005

spermatozoa varied with the constitution of the eggs, although the alternatives were provided by the same female. In other words, conjugation was selective. Having already dismissed the possibility of selective retention because of the results of type 3 control matings, the manner of conjugation here was presumably by selective penetration.

The matter can be examined more closely by studying individual families. As none was large, no single family could be expected to give statistically conclusive results; yet it is not desirable to pay attention to results that were almost certainly due to vagaries of chance. Somewhat arbitrarily, therefore, further attention is confined to those families whose ratios departed far enough from expectation that their likelihood of occurrence by chance was less than one-fifth ($P \leqslant 0.2$). Of twenty-seven experimental families, nine were in this class, and the information from these

Table 5. *Segregation in individually aberrant families of progeny expected in equal numbers*

	Parents		Progeny						
Type	♀ × ♂	Expected relation	Tt^e	$T+$	$+$		Conclusion		
4	$Tt^e \times +t^e$	— : r_T : r_t	—	9	21			$s_T >$	s_t
			—	8	16			$s_T >$	s_t
5	$+t_e \times Tt^e$	r_t : r_+ : —	4	0	—	s_+		$>$	s_t
			0	5	—	s_+		$<$	s_t
			13	2	—	s_+		$>$	s_t
6	$T+ \times Tt^e$	s_T : — : s_+	9	—	18	s_+	$>$		s_T
			0	—	4	s_+	$>$		s_T
			28	—	47	s_+	$>$		s_T
			23	—	36	s_+	$>$		s_T

is set out in Table 5. (Irrelevant phenotypes are not included.) It is observed that, with only one exception, the relative values of the s's throughout the three types of mating fell into a consistent pattern; namely, $s_+ > s_T > s_t$.

The following conclusions are drawn.

1. The great majority, about 84%, of effective spermatozoa from Tt^e and $+t^e$ males transmitted t^e.

2. Maternal genes in matings of type 3 ($T + ♀ \times +t^e ♂$) were equally represented in the progeny and neither gene was selectively retained in the egg nucleus. Unfortunately, as two of the classes of progeny were phenotypically alike ($+t^e$ and $+ +$ are both normal-tailed) these matings provided no direct information about selective penetration.

3. In other matings with heterozygous t^e males, in which three products of conjugation were distinguishable and the fourth lethal, the one genotype of spermatozoa that formed viable combinations with both eggs conjugated with different numbers of them. The missing lethals did not admit direct evidence on the kind of conjugation, but as type 3 families did not display selective retention, it is presumed that these anomalous conjugations involved selective penetration.

4. In this case, since maternal alleles enter the fertilized eggs equally, $(s_T + r_T) = (s_+ + r_+) = (s_t + r_t) = \frac{1}{2}$. Using the data of Table 4 to estimate the numbers of

conjugations of t^e spermatozoa with preferred and avoided eggs, it appears that 55% of t^e conjugations were with preferred eggs, 45% with avoided eggs.

5. The T-locus is probably near a centromere. This conclusion follows from the greater likelihood that portions of chromatids are exchanged between bivalents as distance from the centromere increases. For any included locus, each exchange in oogenesis means that heterozygous alleles will be conveyed together into the products of the first division, and that another egg will be heterozygous when uniting with the spermatozoon. Thus there are few opportunities at distal loci for penetration to be selective. On the other hand, the absence of exchanges proximal to the centromere ensures complete segregation of alleles in this region, complete differentiation of the eggs, and every opportunity is given to selective penetration.

6. DISCUSSION

The Mendelian expectation of offspring from heterozygous parents is $1 : 1 : 1 : 1$. T, $+$ and t^e segregate from females, and T and $+$ (but emphatically not t^e) segregate from males to give progenies agreeing closely with this ratio. Whereas most departures from Mendelian expectation are attributed to phenotypic overlapping or irregular viability, and underlying gametic ratios are assumed to be perfect, this hypothesis is precluded from explaining anomalous segregation of t^e from males. Thus, when heterozygous t^e males are avoided, segregation is perfect for genetically similar offspring to theirs. Gametic anomalies are therefore implicated, and two have been found.

First, t^e is transmitted by spermatozoa of heterozygous males in far greater frequency than either allele. The consequences of this well-known phenomenon have been fully discussed for t genes in general by Dunn (1953) and for the t^e gene in particular by Bateman (1960). It need only be noted here that delays in mating so profoundly affect the transmission of other t alleles (Braden, 1958) that it cannot reflect the numbers of spermatozoa at gametogenesis, and is most likely produced by selective sperm transport inside the female genital tract.

Second, the new observation was made that unequal numbers of t^e spermatozoa fertilized alternative eggs of heterozygous females. It is remarkable that this unique test of the randomness of gametic conjugation conflicts with that usually accepted assumption. However, until selective conjugation is described for other t alleles as well, its occurrence here cannot begin to have general significance.

Nevertheless, the selective conjugations of t^e spermatozoa are particularly relevant to the previously unexplained frequency of $+t^e$ animals in the original laboratory stock (Bateman, 1960). Mice were of two kinds: homozygous normals ($++$) and heterozygotes ($+t^e$). In matings with $+t^e$ males, the proportion, P, of t^e spermatozoa among those partaking in fertilization was estimated to be 0.87, from which it was expected that 0.62 animals would be heterozygous. The actual figure was much higher than this, and was 0.80. These figures were highly discrepant ($P \ll 0.001$). However, it was not then realized that the description of fertilization was incomplete when stated in terms of the overall parameter P and that it was necessary to know the frequencies in which each zygote was formed. Hence the

current description in terms of selective conjugations between t^e spermatozoa and + eggs (s_+), t^e spermatozoa and t^e eggs (s_t); and of residual conjugations between + spermatozoa and + eggs (r_+), and + spermatozoa and t^e eggs (r_t). Previously, the frequency of t^e's entry into zygotes via spermatozoa was judged from fertilizations of T eggs ($P \equiv s_T$), but present evidence shows that t^e spermatozoa made more zygotes with + eggs and fewer with t^e eggs ($s_+ > s_T > s_t$). Both departures would conserve the t^e gene and raise the expected number of heterozygotes. Indeed, it is probable from conclusion 4, that t^e spermatozoa fertilized 10% more + eggs than originally estimated (i.e. 96%, instead of 87%), and 10% fewer t^e eggs (i.e. 78%). If these figures are applied to the original population, assuming random mating and 80% heterozygotes, it will be found that 81% of surviving animals of the next generation are expected to be heterozygotes. Thus it is entirely possible to account for the frequency of lethal heterozygotes in the original laboratory population by the observed results of selective transport of spermatozoa and of their subsequent selective conjugation (penetration) with the eggs. One wonders to what extent these are actually related aspects of selective fertilization. It is conceivable that t^e and + (or T) spermatozoa reach the egg cluster in equal numbers, and that t^e spermatozoa are generally superior at getting into the eggs through the enclosing cumulus oophorus, but are especially superior at getting into + eggs. In this case, the distinction between transport and penetration would be extremely subtle. In any case, the high frequency of lethal heterozygotes in the original laboratory population is accounted for in terms of gametic selection alone without recourse to wide variations in parental fitness other than implicated by the death of $t^e t^e$ offspring. Usually in animal populations gametes are credited with replicating genes perfectly in the same frequencies that they occurred in the parents, and selection is regarded as wholly zygotic. The position taken by Dunn and his collaborators (Dunn, 1953; Dunn & Gluecksohn-Waelsch, 1953; Dunn & Suckling, 1956; Dunn, 1957; Dunn, Beasley & Tinker, 1958) regarding the incidence of tailless-lethals in wild populations is intermediate in the sense that they are looking for explanations using both gametic and zygotic selection.

SUMMARY

Advantage was taken of a unique situation to test, with adequate controls, the assertion that male and female gametes conjugate at random. The data concern the aberrant locus T of the mouse, at which there are a number of t alleles that enter into the majority of effective spermatozoa of males heterozygous for one of them. Segregation in females is normal. Evidence is presented for one of these t alleles, tailless-Edinburgh (t^e), that conjugation between gametes was not at random when this gene was present in spermatozoa. When a choice of eggs was presented by heterozygous females, tailless-Edinburgh spermatozoa united more frequently with normal than with brachyury eggs and more frequently with brachyury than with tailless-Edinburgh eggs. The relevance of this finding is discussed in relation to expected equilibria of t alleles in closed populations. Other forms of selective fertilization are discussed and their genetic consequences compared.

Q

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