THE DIFFERENCE IN SPERM-MORPHOLOGY IN DIFFERENT STRAINS OF MICE

By

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Introduction

A spermatozoa has two important functions, one in the process of reproduction and the other in carrying on the heredity to the posterity. Its size and shape are dissimilar by genera or species and this dissimilarity itself is a hereditary character.

The mammalian spermatozoa have, of course, specificity, and we have many reports on its size, but only few on the morphological difference in species, subspecies or strains.

Blake (1) observed the difference in breeds of bull spermatozoa. According to his research, the head-widths of sperms of Friesian and of Shorthorn bull is wider than that of the Jersey bull. Sometimes, the Hereford and the Ayrshire bull are held distinct from the Jersey bull; the widths of sperm-heads is reported to be a little wider in Hereford than in Jersey bull.

The sperm-head of *Muridae* has a unique hook-shaped form and highly specialized type by species. Friend (7) conducted a comparative study on the form of spermatozoa of British *Muridae* and Monma (15) observed mainly the morphological characteristics of the sperm-head of Japanese *Muridae*; both studied the difference by genera and species and the relationship. Takahashi (23) recently conducted a comparative morphological study of the sperm-head of several kinds of *Muridae* in U.S.A. According to the reports of Friend (7) and Monma (15), it is reported that the form of the sperm-head of mice (*Mus musculus L.*) is remarkably different in comparison with that of other genera of the same family, but neither wrote on the difference by interspecies or interstrains.

In this report, the author has observed first whether there is difference

by interstrains of mice in the size of spermatozoa. The object lies in getting a strain of experimental animals suitable for experimental genetics concerning the size of spermatozoa. I think this means acquiring the facility of studying and examining the relation between the fertility and the hereditary function of spermatozoa in domestic animals by making use of an experimental animal. It will be of great advantage if we can use a small experimental animal like the mouse.

In general, an anomaly of testis or spermatozoa is strongly connected with the fertility of the animal, and at the same time, this anomaly sometimes becomes a hereditary character. Lagerlöf (14) suggests that the reproductivity is related with heredity, reporting that testicular hypoplasia which is incapable of spermatogenesis among Polled Swedish breed of cattle showed low frequency, at first, but increased to about 30 percent in 1935. Erikson (5, 6) made it clear that a autosomal simple recessive inheritance comes into play. Blake (1) said that there are many spermatozoa with narrow head and returned-tail in a certain family of the Jersey bull; their reproductive ability is low and they appear in their posterity by inheritance. Hancock (11) reports on a Friesian bull in which headless spermatozoa appear in high ratio, Hancock and Rollinson (9) say that this is attributed to autosomal recessive inheritance. Such a Friesian bull with headless spermatozoa reported by Hancock was recorded for the first time in the Netherlands in 1943; since his report, such bull were found respectively by Teunissen (24) in the Netherland, by Blom (2) in Denmark, by Rollinson and Makinson (20) in England. This problem also was studied by Bretschneider (3) in the Netherlands, Hancock (10, 11) proposed a technique to make spermatozoon specimens from such a bull. A cytological study in such bull spermatozoa has been conducted by Slizynska and Slizynski (22). They say that such abnormality of sperm-head are associated with vacuole formation occurring during the spermatogenesis and have nothing to do with structural change in chromosomes. After an examination of 17 Holstein bulls with hereditary sterility that have typically abnormal spermatozoa, Donald and Hancock (4) reported that those bulls, with one exception, were sons or grand-sons of a common ancestor A and originated in a Friesian bull imported from the Netherlands to England in 1936, and such a gene probably existed in England before this importation. They concluded that this hereditary sterility is due to recessive gene. The bull A was heterozygous in respect of this recessive gene and the other gene might have originated in the daughters of a common ancestor B.

Reproductive failrue due to such an abnormality of spermatozoa is related to their hereditary function.

Besides the observations on the difference in size of spermatozoa, I

observed the difference of the percentage of abnormal spermatozoa in the interstrain of mice. If the fertility due to abnormality of spermatozoa (percentage of abnormal spermatozoa) is related withe heredity, we can study this problem in domestic animals, using small experimental animals.

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Materials and Methods

1. Materials

The strains and the origins of the mice as materials were as follows:

ss-strain

Origin: U.S.A. 406th Unit $\xrightarrow{(1950)}$ Mr. Umemori (Kasukabe Town, Saitama Prefecture) $\xrightarrow{(1951)}$ The Laboratory of Animal Breeding, Faculty of Agriculture, Tohoku University.

Genetic constitution : ccSSAABB (12).

Character : Strong, docile and highly reproductive; albino.

aa-strain

Origin: Ditto.

Genetic constitution : ccSSaaBB (12).

Character : Strong, very sensible, agile, and prolific; albino.

kk-strain (*Kasukabe* strain)

Origin : Kasukabe Town $\xrightarrow{(1949)}$ Our Laboratory.

Character : Docile, weak in reproductive power and became extinct after the end of this observation.

dd-strain

Origin : Institute for Infections Deseases, Tokyo University $\xrightarrow{(1953)}$ Our Laboratory.

Genetic constitution : ccSSaaBB (12).

Character : Strong and docile, quick-growing, and heavy on maturing; albino.

rr-strain (*Red* strain)

Origin: An animal dealer (Sendai City) $\xrightarrow{(1949)}$ The Pathological Laboratory, Tohoku University, Faculty of Medicine $\xrightarrow{(1950)}$ Our Laboratory.

Genetic constitution : CCSSaabb (12).

Character : Cannot be called docile, not very prolific nor quick-growing; chocolate-furred.

All the strains were bred by sib-mating.

2. Methods

The observations of abnormal spermatozoa were carried out in the same manner as in my previous report (16, 17).

Results and Discussion

1. The difference of size of sperm-head by strains

As I had learned that the sperm-heads of *ss*-strain is larger than that of *aa*-strain during observation of spermatozoa of mice of the above strains, I measured and compared the size of the sperm-head in these two strains. At the same time I made a similar measurement with sperm-head in normal and reciprocal F_1 hybrids between the two strains. The sperm-heads were measured lengthwise, as shown in Fig. 1. 20 spermatozoa were thus measured per sample.

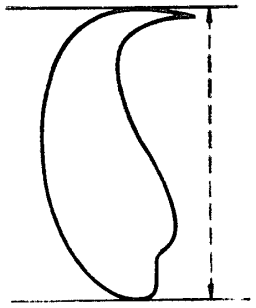

Fig 1. The measured length of sperm-head.

The length of sperm-head of the two strains and their hybrids F_1 are shown in Table 1.

Table 1. The head-length of spermatozoa of *ss*-strain, *aa*-strain and their hybrids F_1.

Mating ♀	Mating ♂	No. of mice	Head-length $\bar{x}$	Head-length s
ss	*ss*[1]	15	8.11	0.13
aa	*aa*[2]	12	7.35	0.19
ss	*aa*[3]	15	7.82	0.11
aa	*ss*[4]	17	8.19	0.15
Singnificance of difference		1 : 2	<0.001	
		1 : 3	<0.001	
		1 : 4	<0.2	
		2 : 3	<0.001	
		2 : 4	<0.001	
		3 : 4	<0.001	

The head-length of *ss*-strain is clearly larger than that of *ss*-strain. The head-length of the hybrid $aa \times ss$-F_1 is smaller than that of *ss*-strain but larger than of *aa*-strain. In $aa \times ss$-F_1, is nearly equal to that in *ss*-strain but is larger than that in *aa*-strain. In the reciprocal hybrid F_1, the head-length of $ss \times aa$-F_1 is smaller than that of $aa \times ss$-F_1.

Thus, the head-length of spermatozoa is larger in *ss*-strain in *aa*-strain. I consider this difference to represent hereditary characteristics in the size of spermatozoa in the two strains. In a case of $ss \times aa$-F_1, the head-length

of spermatozoa of F_1 may be said to represent the mean between the values of the parent strains. On the other hand, in the case of $aa \times ss$-F_1 it may be said that the value is determined by the dominant character in *ss*-strain. At any rate, it is not easy to understand why the head-length of spermatozoa comes out different in F_1's of $ss \times aa$ and $aa \times ss$, but I am inclined to attribute this phenomenon to a difference in the hereditary formula.

A more detailed experimental examination may be required to analyse the mechanism of heredity about size of the spermatozoon, but it may be said that mice of these two strains have characteristics befitting materials of study for experimental genetics on the size of spermatozoon.

2. Classification of abnormal spermatozoa of mice

We have many reports on the morphological classification of abnormal spermatozoa in man and domestic animals, but only few reports on the abnormal spermatozoa of mice or rats. I took the current classification system in domestic animals into reference and followed my previous method (16, 17) in observation and classification of abnormal spermatozoa of mice. I made reference to the observations of Friend (7) and Monma (15) about the normal of mouse spermatozoa sampled from the cauda epididymidis. The method of observation for abnormal spermatozoa was the same as used previously (16, 17).

I classified the abnormal spermatozoa of mice as shown in Table 2 and counted the malformations as shown in Fig. 2.

Table 2. Classification of abnormal spermatozoa in mice

- Abnormal spermatozoa
  - Whole form
    - Abnormal head
      - Deformed form
      - Dwarf form
      - Malformed form
      - Giant form
      - Multiple form
      - Other form
    - Abnormal neck
    - Abnormal mid-piece
    - Abnormal connecting piece
    - Abnormal tail
    - Combined form
    - Bundled form
  - Separated form
    - Tailless form
    - Headless form

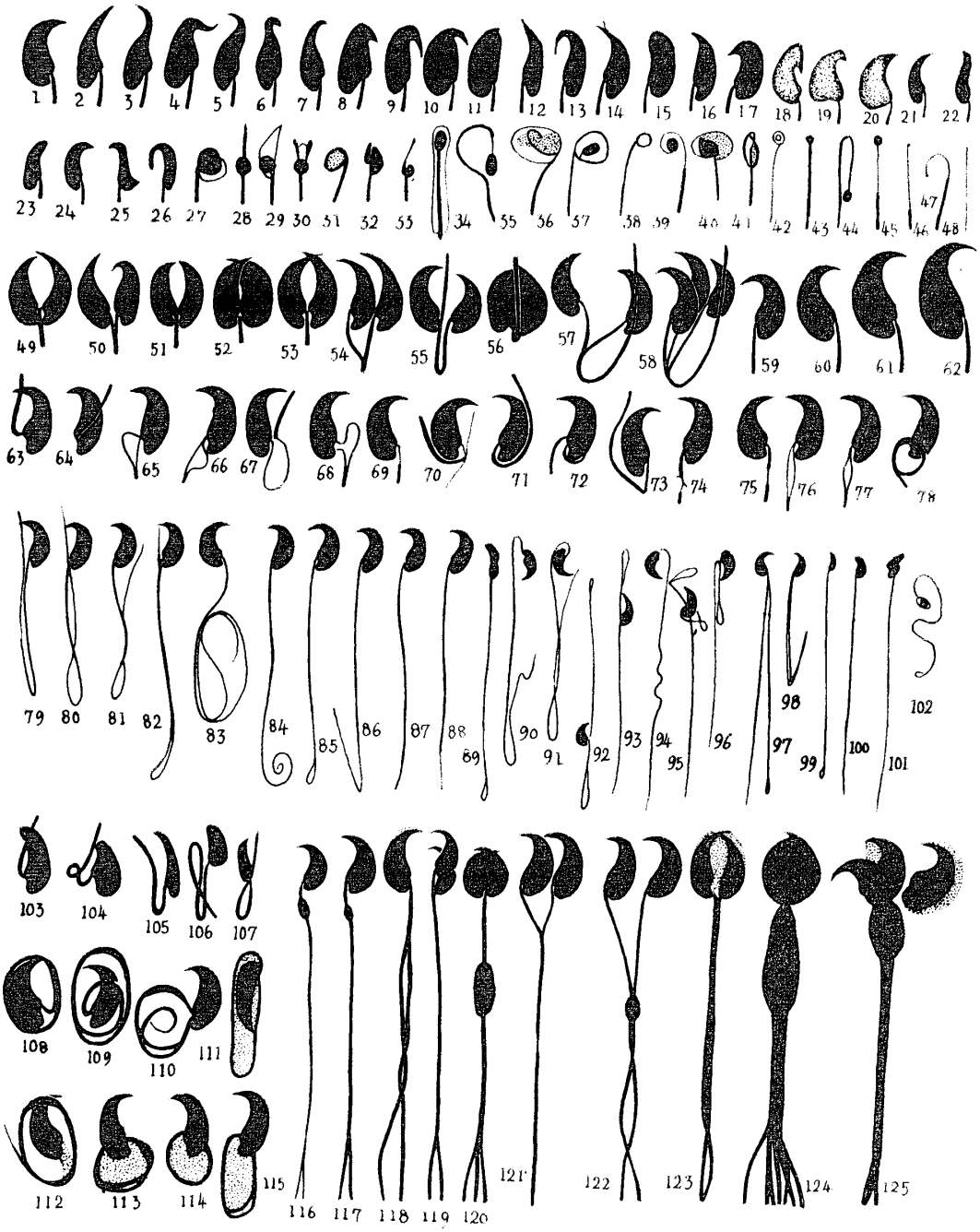

Fig. 2 Types of abnormal spermatozoa.

1) **Abnormal head**

The normal form of the sperm-head of mice is flat and hook-shaped, showing a specific right-left asymmetry, as illustrated in Fig. 2 No. 1. The following figures (Nos. 1-78) are in the same scale of magnification.

Deformed form: Nos. 2 through 17. Nos. 18-20 show abnormal staining at the same time indicating poverty in chromatin; these were most frequent in the *Kasukabe* strain. Deformed head was the most frequent type among

the abnormal head.

Dwarf form: As illustrated in Nos. 21-26. No. 21 shows the normal size. These may represent heteroploids.

Malformed form: Nos. 27 through 48. It is very low in proportion among abnormal heads, but shows many variations. It occurs mostly in oligopyrene or apyrene sperms. Such sperms were seen in rats and specially frequently in experimental cryptorchidism of rats (16). No. 48 is a hairy spermatozoon with the head reduced to a point.

Multiple form: As illustrated in Nos. 49-58. Nos. 49-57 show double-head. No. 58 shows triple-head.

Giant form: As shown in Nos. 60-62. That in No. 59 is of normal size, and we may take those in Nos. 60-62 as representing a diploid, a tetraploid and a octoploid, respectively. Breadth ratio of the sperm-head was 8 : 4 (μ) in No. 59, 9.6 : 4.8 in No. 60, 10.4 : 5.3 in No. 61 and 11.2 : 5.6 in No. 62. These were frequent, in the *Kasukabe* strain. It may be justifiable to call these giant spermatozoa polyploid spermatozoa. Morimune (19) reports that such sperms always exist in normal young or aged rats and he has observed some also in starved rats. As to the cause of appearance of giant spermatozoa, he says that at first, the spermatogenic cells in the seminiferous tubules is suspended and then giant spermatozoa are formed. Friend (7) has observed this process in English *Muridae* and Monma (15) in *Apodemus semotus* Thomas.

Other forms : Broken, bent or injured heads.

2) **Abnormal neck** :

Bent (Nos. 63-64), capsule formation (Nos. 65-68), filiform (No. 69) and brocken neck (No. 70).

3) **Abnormal mid-piece** :

Coiled (Nos. 71-72), bent (No. 73), broken (No. 74), filiform (No. 75), hugeness (Nos. 76-77) and torsion (No. 78).

4) **Abnormal connecting piece** :

Types shown in Nos. 79-81 are the most frequent. These photographs are in lower magnification than the preceding. This abnormality rapidly increased in the rat supplied with protein-free rations (17).

5) **Abnormal tial** :

Magnification as low as in 4) above. These are coiled (Nos. 82-85), bent (No. 86), disappearance (No. 87) and filiform (No. 88) of the posterior part. These abnormality appeared extremely often in rats fed with supplied protein-free rations (17) and increased in rats injected with female hormones (18).

6) **Combined form** :

Abnormality in two or more parts occurring in the same sperms, as shown in Nos. 89-115. The magnification in Nos. 89-102 is lowered even below that of 4) and 5); in Nos. 103-115 it is about the same as in 3) above.

7) **Bundied form :**

Various bundled spermatozoa are shown in Nos. 116-125. Sometimes, we may see diploids in or polyploids them, Similar bundled sperms were observed in experimental cryptorchidism in rats (16). Fairly many of them exist in mice. These were found in *kk*- and *ss*-strains, but never in spermatozae of any other mice.

8) **Tailless form :**

Illustrations omitted. It may be that they are either tailless from the first or have been deprived of their tail during their formation, but it is difficult to discriminate between them by form.

9) **Headless form :**

It is difficult to discern between the original headless sperms and the spermatozoa deprived of the head. Illustration omitted.

10) **Presence of the protoplasmic drops :**

I do not subscribe to the opinion for treating immatured spermatozoa with a protoplasmic drop on the neck or the mid-piece as abnormal spermatozoa.

In abnormal spermatozoa of mcie, there are many kinds of abnormal head. It seems that this is due to the unique form of mouse spermatozoa, as in rats. Giant sperm-head (polyploid spermatozoa) were frequent in the *Kasukabe* strain (*kk*-strain); this is perhaps a characteristic of this strain. The same may be said of bundled spermatozoa in *ss*- and *kk*-strains.

3. Comparison of rates of abnormal spermatozoa in mice of different strains

1) Comparison in mice of different strains

Table 3. Comparison of rates of abnormal spermatozoa in mice of different strains

	Year	Strains	No. of mice	Kinds and distribution of percent of abnormal spermatozoa					Total %	Significance of differences
				Head	Neck	Mid-piece	Tail	Combined		
I	1952	*ss*	8	4.66	0.90	5.66	7.66	5.55	24.43	$P<0.001$
		aa	5	1.28	0.24	1.32	3.00	0.96	6.80	
II	1952	*ss*	10	4.02	1.12	4.89	8.75	6.98	25.76	$P<0.02$
		aa	9	2.76	0.40	2.21	3.24	1.97	10.59	
III	1953	*ss*	7	6.06	0.94	4.24	7.37	6.36	24.97	$P<0.01$
		aa	3	1.83	0.10	1.00	0.83	1.10	4.90	
IV	1953	*ss*	15	3.70	0.44	7.09	11.13	6.49	28.79	$P<0.01$
		aa	12	2.60	0.33	3.91	1.28	1.30	9.42	
V	1953	*ss*	10	4.12	0.90	3.27	5.05	6.45	19.79	$P<0.001$
		aa	12	0.97	0.49	1.13	1.03	0.57	4.19	
VI	1955	*ss*	10	3.56	0.59	4.81	5.25	5.45	19.66	$P<0.001$
		aa	5	0.92	0.76	3.52	2.34	1.82	9.36	
VII	1957	*dd*	5	2.04	0.76	5.44	13.64	8.60	30.48	$P<0.01$
		aa	8	1.65	0.65	4.30	2.10	1.18	9.88	
VIII	1957	*rr*	6	3.00	0.17	3.00	10.00	2.50	18.67	$P=0.05$
		aa	8	1.65	0.65	4.30	2.10	1.18	9.88	
IX	1953	*kk*	4	18.45	1.50	3.05	2.43	5.78	31.21	$P<0.001$
		ss	4	5.60	0.52	2.97	2.93	4.08	16.10	
X	1955	*dd*	5	6.72	1.14	3.74	5.96	4.14	21.70	
		ss	10	3.56	0.59	4.80	5.25	5.45	19.66	
XI	1956	*dd*	7	1.80	0.30	3.29	13.91	5.60	24.90	
		ss	8	1.82	0.09	6.76	17.41	5.54	31.62	

Table 3 shows the result of comparative study of abnormal spermatozoa of two strains each, in chronological order (1962-1956), of the five strains *ss*, *aa*, *dd*, *rr* and *kk*. The source of the data is a series of my study on the heterosis (unpublished data).

As clear from Table 3, that the frequency of abnormal spermatozoa in *aa*-strain is markedly lower than that in *ss*-strain (I-VI), *dd*-strain (VII) or *rr*-strain (VIII). This low rate of abnormal sperms is a reproductive characteristic and seems to be at the same time a hereditary character of this strain. The rate is not different between *kk*- and *ss*-strain (IX) or *dd*- and *ss*-strains (X and XI).

2) Distribution of the rates in mice of different strains

The distribution of the rates of abnormal sperms of mice of the five strains, *ss*, *aa*, *dd*, *rr* and $C57/_{BL}$ is shown in Fig. 3. The data except those concerning $C57/_{BL}$ include those cited in the preceding table. In this figure, the rate is lower and the standard deviation (s) is also lower in *aa*-strain than in any other strain.

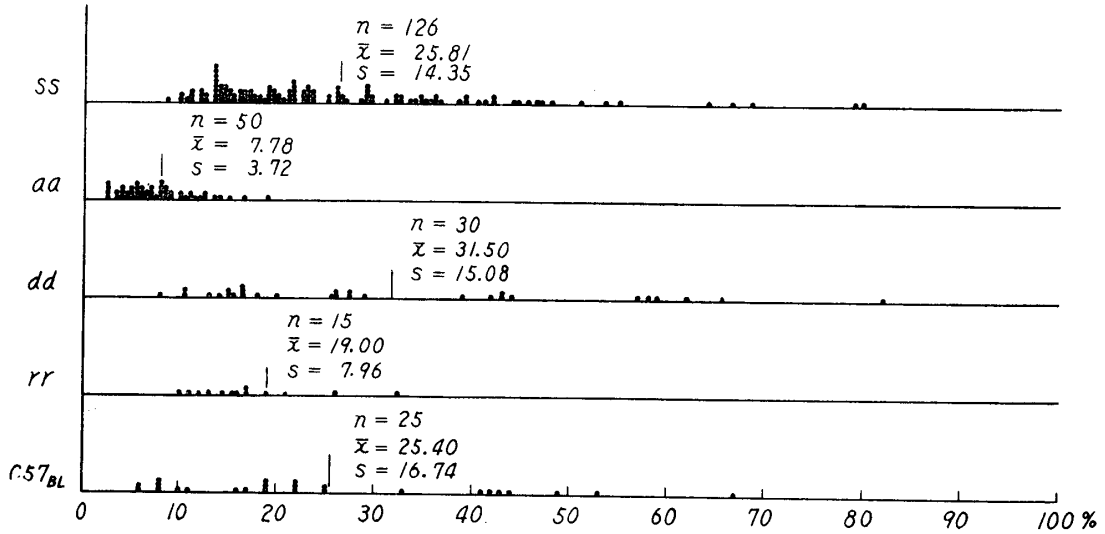

Fig 3. Distribution of rate of abnormal spermatozoa in 5 strains of mice.

3) Rate of abnormal spermatozoe in *ss*-, *aa*-strains and reciprocal hybrids F_1

I made comparison of the rate of abnormal spermatozoa in hybrids F_1 obtained by reciprocal crossing between *ss*-strain with high rate and *aa*-strain with lower rate of abnormal spermatozoa. The results are shown in Table 4. This experiment was done at the time of mating shown in Table 3 (IV).

The rate of abnormal spermatozoa in the crossbreds F_1, reciprocally, is neither equal to that of any inbreds strain nor falls between those of the

parent strains but is lower than in any of the parents strains. I am inclined to believe that the lowness of the rate in hybrids F_1 is partly an heterosis effect, but also note the following: 1) The rate in reciprocal hybrids F_1 always approximates not that in *ss*-strain with higher rate but that in *aa*-strain with lower rate, and 2) the level of the rate of abnormal spermatozoa is different in the hybrids F_1 of two kinds, i.e., lower in $ss \times aa$-F_1 than in $aa \times ss$-F_1. The phenomena 1) and 2) are also seen in reciproaal $ss \times aa$, $rr \times aa$, $dd \times aa$ and reciprocal $aa \times ss$-F_1-F_{10} too the rate is low, suggesting that the low rate as a hereditary character of *aa*-strain predominantly appears in the offsprings.

Judging from these findings, it may be inferred that the rates of abnormal spermatozoa in *ss*- and *aa*-strains are specific to these two strains, respectively, and in hereditary effect, the rate in *aa*-strain is stronger than that in *ss*-strain, as is suggested by the approximation of the rate in the hybrids F_1 to that in *aa*-strain and the lowness of the rate in F_1 and later generations of the hybrids between the two strains. The difference in the mode of appearance of the rate in hybrids F_1 of $ss \times aa$ and $aa \times ss$ suggests that the hereditary constitution comes out differently by the reciprocal of mating, owing to the difference in the hereditary function in the two strains concerning the malformation of spermatozoa.

Table 4. Rate of abnormal spermatozoa in mice of two strains and their reciprocal hybrids F_1.

Mating ♀ × ♂	No. of mice	Kinds and distribution of per cent of abnormal spermatozoa					Total %
		Head	Neck	Mid-piece	Tail	Combined	
ss × *ss*[1]	15	3.70	0.44	7.09	11.13	6.49	28.76
aa × *aa*[2]	12	2.60	0.33	3.91	1.28	1.30	9.42
ss × *aa*[3]	15	0.77	0.21	1.68	1.37	0.63	4.66
aa × *ss*[4]	17	0.98	0.30	2.44	1.09	0.84	5.65

Signicicance of differences (Total %)

1 : 2	<0.001
1 : 3	<0.001
1 : 4	<0.001
2 : 3	<0.001
2 : 4	<0.05
3 : 4	<0.3

Conclusion

In mice (*ss*- and *aa*-strain) the size of the sperm-head is different ($ss > aa$), and I think this is a hereditary character. The size of the sperm-head in hybrid F_1 by interstrain crossing of mice of these two strains is different in

ss×*aa*-F_1 and *aa*×*ss*-F_1. I think that this is caused by a difference in heredity related with the size of spermatozoa of the two strains.

The abnormal spermatozoa of mice may be classified like those of other domestic animals, and are characterised by the numerous kinds of abnormality of their head. Besides, giant spermatozoa (polyploid spermatozoa?) and bundled spermotozoa were observed, particulary frequently in certain strains.

In mice of the six strains, *ss*, *aa*, *dd*, *rr*, *kk* and *C57/BL*, the rate of abnormal spermatozoa is distinctly lower in *aa*-strain than in any other strain; this low rate in *aa*-strain may be said to be a hereditary character of this strain. In the case of the interstrain crossing of mice of *aa*-strain and of other strains (*ss*-strain), the rate of abnormal spermatozoa in the hybrids F_1 is reduced by the heterosis effect, but the reduction is not equal in F_1 of *ss*×*aa* and *aa*×*ss*, being larger in the latter. I think this phenomenon is caused by a difference of hereditary factors related with the abnormality rate in the two strains (*ss*<*aa*).

The mice of these strains are thus apparently suitable as small experimental animals for studying the hereditary and the reproductive functions of domestic animals.

Summary

Upon comparative measurements of the head-length of spermatozoa, morphological classification and a comparison of the rates of abnormal spermatozoa, of mice of several strains, the following results were obtained:

Size of sperm-heads

1) The head-length of spermatozoa of *ss*-strain is 8.19μ and that of *aa*-strain is 7.35μ. This difference in size is a hereditary character.

2) The head-length of spermatozoa of hybrid F_1 is different by reciprocal crosing: In *ss*×*aa*-F_1 it is 78.2μ, but in *aa*×*ss*-F_1 it is 8.19μ. I think this is based on a difference in the genes concerned with the size of spermatozoa in the two strains.

Abnormal spermatozoa

3) The abnormal spermatozoa may be classified into abnormalities in the head, in the mid-piece, in the tail, combined forms and separated spermatozoa (spermatozoa without tail or head). Giant (polyploid) spermatozoa and bundled spermatozoa are seemingly specific to certain strains.

Rate of abnormal spermatozoa in different strains of mice

4) Of *ss*, *aa*, *dd rr*, *kk*, and *C57/BL* strians, *aa*-strain shows a remarkably low rate of abnormal spermatozoa. I think that this is a characteristic of this strain.

5) The rate of abnormal spermatozoa in hybrid F_1 between the *ss*-strain

with high rate and the *aa*-strain with low rate is lower than that of either parent strain. This rate is different by the way of crossing ($ss \times aa$-$F_1 < aa \times ss$-F_1).

6) I think this is partly due to heterosis effect, and that the heredity in respect to the rate of abnormal spermatozoa is different in these two strains, resulting in the difference of hereditary constitution between the $ss \times aa$ and the $aa \times ss$-F_1.

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