Studies on Genetics and Breeding of the Bangladesh Musk Shrew, *Suncus murinus*

(バングラデシュ産スンクス, *Suncus murinus*, に関する遺伝育種学的研究)

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CONTENTS

Chapter I.

General introduction

The musk shrew (*Suncus murinus*) is one of the insectivore species. It was attempted to domesticate the shrew as a unique laboratory animal in the early 1970s at the Laboratory of Animal Genetics, Nagoya University, Japan (Kondo, 1985a; Oda and Kondo, 1977). The first laboratory strain, NAG, has been established from wild shrews captured in Nagasaki, Japan in 1973 (Oda and Kondo, 1976). After that, wild shrews were frequently captured on Okinawa Island, Izenajima Island, Tokunoshima Island, etc., Japan (Oda, 1985a; Oda and Shigehara, 1978), and various strains have been established from them (Iseki, 1985; Namikawa *et al.*, 1985). Many investigators have revealed many biological characteristics of these Japanese shrew strains (Kondo, 1985b).

However, wild shrews are widely distributed throughout the East, Southeast and South Asia, and East Africa (Oda, 1985a). They show extreme geographical variations both in adult body weight, varing from about 45 g to about 170 g in males and from about 25 g to about 100 g in females (Hasler *et al.*, 1977; Louch *et al.*, 1966; Namikawa *et al.*, 1985; Oda and Kondo, 1976; Tsubota *et al.*, 1986), and in diploid chromosome number, ranging from 2N=30 to 2N=40 (Andō *et al.*, 1980;

Duncun *et al.*, 1970; Manna and Talukdar, 1967; Obara and Miyai, 1981; Yong, 1971; Yosida, 1982). The coat hair is colored either light gray or dark gray (Dryden, 1968; Hasler *et al.*, 1977; Ishikawa *et al.*, 1989). In addition, based on restriction endonuclease cleavage types of mitochondrial DNA (mtDNA), the wild shrew population in Bangladesh is differentiated from those in Japan, Indonesia, and Sri Lanka to the extent which can be compared to intersubspecific differences in *Mus musculus* (Yamagata *et al.*, 1990).

It is very much important to establish many kinds of shrew strains which are different in their genetic characteristics, like inbred or mutant strains of laboratory mice (Lyon and Searle, 1989). This is the first step for the laboratory shrew becoming a true "experimental animal" which contributes to the progress of life science. Recently a unique shrew strain, SRI, has been developed from wild shrews in Sri Lanka, which is characterized by having 30 chromosomes in diploid, being the smallest number in this species (Ishikawa *et al.*, 1989). A cream coat-color mutation controlled by a single autosomal recessive gene (symbol *cr*) was found in the descendant from Indonesian wild shrews, which were carriers of this gene (Iseki *et al.*, 1984). These two examples provide the evidence that wild shrew populations, which have a great deal of genetic variations as mentioned above, play a very much

important role as potential sources of novel genetic variants or genes in the laboratory shrew.

Recently wild shrews were captured in Bangladesh, the place in which a collection of shrews had not been made so far. From a part of the shrews captured, I have succeeded in developing a new laboratory strain, BAN, in my laboratory. The BAN shrew is morphologically characterized by one of the largest animals in this species.

The objective of the present thesis is to describe a historical account of the origin of the BAN strain and its genetic characteristics with special reference to chromosomes, reproductive isolation mechanism, postnatal growth, and a morphological mutant. Chapter II describes morphological and reproductive characteristics of the Bangladesh wild shrews, from which the BAN strain originated. Chapters III to VII deal with the genetic properties of the BAN strain itself in comparison with the other strains previously established from wild shrew populations. In Chapter III, karyotypic characteristics of the BAN shrew are described in comparison with those of other strains from Japan and Sri Lanka, by means of the conventional Giemsa staining method. Chapter IV is concerned with the influence of body weight difference between sexes on mating success in reciprocal crosses between the BAN (large size) and NAG (small size derived from Nagasaki,

Japan) strains. Chapters V and VI are concerned with the genetic difference in postnatal growth between the large BAN shrew and the small NAG shrew. Chapter VII provides an estimate of the minimum number of genes contributing to the difference in the adult body weight of the two strains. As a fact of genetic variability within the BAN strain, Chapter VIII presents genetic analysis and phenotypic characteristics of a new mutation causing the kinky coat discovered in the strain.

Chapter II.

Morphological and reproductive characteristics of wild musk shrews collected in Bangladesh, and development of a laboratory strain (BAN) derived from them

INTRODUCTION

Wild musk shrews were collected in Bangladesh and a part of them were introduced into my laboratory in order to establish a new laboratory strain. The present chapter describes morphological and reproductive characteristics of the wild shrews caught in Bangladesh and development of the laboratory strain (BAN) derived from them. This is a historical account of the origin of the BAN strain.

MATERIALS AND METHODS

In the first trapping period, from October through November 1983, 74 wild shrews (37 for each sex) were captured in the campus of Bangladesh Agricultural University, Mymensingh. In the second period, from December 1985 to January 1986, 12 wild shrews for each sex were collected at the same trapping sites by using Sherman traps (7X9X23 cm), with fish, meat chops or insects as baits. The traps were set in the evening. Wild shrews captured were measured for body weight, head-body length and tail length with a beam balance and a slide caliper in the laboratory. After that,

most of the shrews were sacrificed for obtaining their blood and tissue samples. When a female was trapped, she was examined for lactation and pregnancy, and if pregnant, the number of fetuses was counted.

On November 26th 1983, 5 males and 7 females of the shrews collected in 1983 were introduced into my laboratory as the original breeding stock to develop a new laboratory strain (BAN) of the musk shrew. These shrews were maintained in a temperature- and light-controlled room (23-27^{o}C; 14L10D), in which humidity was not controlled. Commercial pellets designed for the musk shrew (Central Institute for Experimental Animals, Kawasaki) and for rainbow trout (Nippon Haigo Shiryo, KK., Tokyo), and tap water were provided as the basic diet *ad libitum*. In addition, an appropriate amount of fresh minced chicken meat, commercial dog food (Nippon Haigo Shiryo, KK., Tokyo) or boiled eggs were provided daily. The shrews were housed individually in a wooden cage (25X25X50 cm) that was bedded with shavings and equipped with a wooden nest box containing paper toweling strips.

After the 12 shrews had been reared for more than 100 days in the laboratory, the body weight, head-body length and tail length were measured with an electric balance and a slide caliper. A female was mated with a male introduced into her cage for one or a few days. The females were examined for parturition every

morning. Gestation period was estimated only from those females mated within one night. Litter size was determined between 10 and 20 days after birth.

RESULTS

Morphological and Reproductive Characteristics of Wild Shrews

All of the wild shrews trapped in Bangladesh were covered with light gray fur. The belly fur was lighter gray in color than the dorsal fur. The skin was pale pink in color. The coat and skin color exhibited slight individual variations.

The tail, head-body and total lengths of all shrews captured are presented in Fig. 2-1. Excluding the shrews reared in the laboratory, the tail length ranged from 5.8 to 13.3 cm in males and from 8.0 to 9.9 cm in females, while the head-body length ranged from 11.4 to 19.6 cm in males and from 12.9 to 17.7 cm in females. The total length ranged from 17.2 to 31.9 cm in males and from 21.1 to 26.6 cm in females. The shrews indicated by the darker parts of the columns in Fig. 2-1 were raised for more than 100 days under laboratory conditions and regarded as fully adult. Then, based on the distribution of tail, head-body and total lengths in the laboratory shrews, the lengths of the adult wild shrews were estimated to be as follows: the tail length was more than 8.5 cm in males and more

than 7.5 cm in females; the head-body length was over 16.5 cm in males and over 15.0 cm in females; and the total length was more than 25.0 cm in males and more than 23.0 cm in females. It was concluded that the shrews collected were obviously of various ages.

Figure 2-2 indicates body weight distribution in the shrews trapped. The male shrews not reared in the laboratory weighed from 32.5 to 147.0 g and the females from 40.8 to 110.0 g. The adult body weight was more than 130 g in males and over 60 g in females. However, it was suggested that since many of shrews captured were weighed several hours or days after being trapped, their body weight might have been decreased by stress or fasting in the trap and during subsequent care. Therefore, the body weight of the shrews trapped may have been underestimated.

Monthly changes in reproductive activities in the shrews trapped are presented in Fig. 2-3. Pregnant and/or lactating females and some young shrews of both sexes were trapped in all months. The data indicate that most of females and probably males have reproductive activities throughout these months at least.

The number of fetuses could be counted in 11 of 13 pregnant females collected (Table 2-1). Of the two females not counted, one had embryos being too premature to count exactly and the other had an abortion. The average number of fetuses was 3.54 (ranging from 2

to 5); the most frequent litter size was 4 at the fetal stage. There was no significant difference in the number of fetuses between left and right uterus (X^2-test, $0.50<P<0.70$).

The Origin of the BAN Strain

Table 2-2 shows body weight, head-body length and tail length of the wild shrews introduced into my laboratory. These shrews were measured after being reared for more than 100 days under laboratory conditions. They were regarded to be fully adult.

Mating records for the wild shrews subjected to laboratory conditions are shown in Table 2-3. Fifty-nine pups were successfully obtained by crossing 7 females with 4 of the 5 males introduced. These pups were regarded as the first generation of a new laboratory strain, BAN. The mean litter size was 3.47. As a result of over-night mating in 10 cases, the gestation period was determined to be between 28 and 30 days.

The BAN strain has been maintained in my laboratory as a closed breeding colony consisting of about 60 individuals at each generation since the original stock was introduced, and efforts to prevent close inbreeding have been made as far as possible. This strain is now at the 14th generation. From the first to the present generation, husbandry of the BAN shrews has been done by the same methods described in *Materials and Methods*, but the laboratory-bred shrews have been housed indi-

vidually in a polycarbonate cage (20X25X40 cm).

DISCUSSION

Wild shrews trapped in Bangladesh usually had a light gray pelage resembling that of shrews collected in Sri Lanka (Tsubota *et al.*, 1986). On the other hand, the coat of Japanese shrews exhibits a dark gray color (Iseki, 1978). Generally the shrew is known to have a coat colored either light gray or dark gray (Dryden, 1968; Hasler *et al.*, 1977).

Many of the Bangladesh wild shrews were found to be reproductively active throughout the trapping period from October to January (Fig. 2-3). In Calcutta, West Bengal, the nearest locality to the present trapping site, Louch *et al.* (1966) reported a relatively high level of reproductive activity of wild shrews throughout the year. Harrison (1955) reported a similar phenomenon for Malayan wild shrews.

The number of fetuses in the pregnant shrews collected in Bangladesh averaged 3.54, ranging from 2 to 5 (Table 2-1). The wild shrews trapped in Nagasaki and Okinawa Island, Japan had 3.67 fetuses on average, ranging from 1 to 6 (Morita, 1964), and 2.2, ranging from 1 to 5 (Oda and Shigehara, 1978), respectively. It is therefore inferred that the musk shrew usually has between 1 and 6 fetuses at one pregnancy.

The gestation period of the Bangladesh wild shrews

housed in the laboratory was between 28 and 30 days. This value closely agrees with the results described in previous reports on this species (Dryden, 1969; Oda and Kondo, 1976).

The body weight of both sexes of shrews from Bangladesh exceeded that of Japanese shrews by more than 2 times (Table 2-4), and these shrews were bigger than Japanese shrews in body size as well (Fig. 2-4). On the other hand, the shrews trapped in Bangladesh were as heavy as those from West Bengal (Table 2-4). It is clear that the Bangladesh shrew is one of the largest animals in this species.

Besides the above-mentioned unique morphological traits, the Bangladesh shrew population is reported to be differentiated from wild shrew populations collected in Japan, Indonesia, and Sri Lanka to the extent which can be compared to intersubspecific differences in *Mus musculus* based on restriction endonuclease types of mtDNA, and surprisingly such a level of differentiation is found between the two of three mtDNA types within the Bangladesh population (Yamagata *et al.*, 1990). In addition, Tsubota and Namikawa (1988) reported that the wild shrews from Japan, Indonesia, and Sri Lanka have two types of alleles at the plasma α-amylase (*Amy-1*) locus in common, whereas the Bangladesh shrews have these common types of alleles and another type of allele never found in the Japanese,

Indonesian, and Sri Lanka shrews. These may mean that the Bangladesh shrews possess higher genetic variability within the population than the other shrews. Yamagata et al. (1987) and Tsubota and Namikawa (1988) verified that such unique genetic properties are conserved in the BAN strain derived from the Bangladesh wild shrews.

From the above facts, it is concluded that the BAN strain is a very unique one characterized by its extreme morphological traits as well as high genetic variability. Hence the BAN shrew can be expected to retain further peculiar genetic characteristics, which will be revealed from the next chapter onwards.

SUMMARY

To establish a unique laboratory strain of the musk shrew with different genetic properties from previously developed laboratory strains, 49 male and 49 female shrews were captured in the campus of Bangladesh Agricultural University from October through November in 1983 and from December in 1985 to January in 1986. The shrews collected were of various ages. They had light gray coats, with slight variation in color. Except for the 12 shrews introduced into my laboratory, the total length and body weight of the shrews ranged from 17.2 to 31.9 cm and from 32.5 to 147.0 g in males, and from 21.1 to 26.6 cm and from 40.8 to 110.0 g in

females, respectively. Pregnant females were found throughout the trapping period, and the average fetal litter size was 3.54 (11 cases). Five males and 7 females of the shrews captured in 1983 were transported to my laboratory. After more than 100 days of laboratory rearing, their total length and body weight averaged 27.6 cm and 147.3 g in males, and 24.6 cm and 81.7 g in females. Their body weight was more than double that of Japanese shrews. The shrews introduced (except for one male) produced a total of 59 offspring, which were regarded as the first generation of the laboratory strain (BAN). Gestation period and average litter size were between 28 and 30 days (10 cases) and 3.47 (17 cases), respectively. The BAN strain has been maintained as a closed breeding colony, consisting of about 60 individuals at each generation, and is now at the 14th generation.

Table 2-1. Number of fetuses found in eleven pregnant female shrews trapped in Mymensingh, Bangladesh.

Individual number for the pregnant females trapped	Number of fetuses		
	Left uterus	Right uterus	Total
'83, October			
I-2	2	2	4
I-4	2	2	4
I-15	2	2	4
I-23	2	3	5
I-24	2	1	3
November			
I-40	1	2	3
I-57	3	2	5
'85, December			
II-7	1	2	3
'86, January			
II-11	3	1	4
II-12	1	1	2
II-15	2	0	2
Total	21	18	39
Number of fetuses per female			3.54

Table 2-2. Body weight (BW), head-body length (HBL), and tail length (TL) of the wild shrews from Mymensingh, Bangladesh, captured in 1983. These animals were the original stock for breeding a new laboratory strain (BAN). Measurements were made after the animals had been reared for more than 100 days in the laboratory, and the females were not pregnant at the time. Trap sites were located in the campus of Bangladesh Agricultural University. Site *B* was around a poultry pen, and sites *A* and *C* were each less than 2 km distant from site *B*.

Individual number	BW (g)	HBL (cm)	TL (cm)	Total length (cm)	Trap site and date
Male-I	132.3	18.1	10.5	28.6	*A* Nov. 14
-II	142.9	18.7	9.1	27.8	*B* 17
-III	150.2	16.9	9.9	26.8	*B* 17
-IV*	141.8	17.0	8.9	25.9	*B* 17
-V	169.3	18.6	10.1	28.7	*B* 17
$\bar{X}\pm SD$	147.3±13.8	17.9±0.9	9.7±0.7	27.6±1.2	
Female-1	71.7	16.1	7.8	23.9	*C* Nov. 16
-2	65.8	15.3	8.9	24.2	*B* 17
-3	82.6	15.9	8.7	24.6	*B* 17
-4	91.0	15.8	9.2	25.0	*B* 18
-5	102.3	15.6	8.9	24.5	*B* 17
-6	75.8	15.9	9.6	25.5	*B* 18
-7	82.4	15.5	8.8	24.3	*B* 17
$\bar{X}\pm SD$	81.7±12.2	15.7±0.3	8.8±0.6	24.6±0.5	

*: Male-IV did not contribute to producing a later generation.

Table 2-3. Numbers of litters and pups obtained by crossing among the wild shrews caught in Bangladesh in the laboratory. Matings were planned so as to obtain offspring from all of the male-female combinations. The number of pups in a litter was recorded between 10 and 20 days after birth. The pups obtained were regarded as the first generation of the laboratory strain of the Bangladesh musk shrews (BAN strain).

		Individual number for wild-caught males					Number of pups per litter in a female
		I	II	III	IV	V	
	1	(3)	(1)	-	-	(3)	2.3
Individual	2	(4)	(4)	-	-	(3)	3.7
number for	3	+	(4)(3)	(5)	-	(3)	3.8
wild-caught	4	-	(2)	(3)	-	-	2.5
females	5	(3)	-	-	-	(4)	3.5
	6	(5)	(5)	-	-	-	5.0
	7	-	+	+	-	(4)	4.0

Total number of pups obtained: 59 (26 males and 33 females)
Total number of pups obtained/litter: 59/17=3.47

Each parenthesis indicates one litter and the number shows the litter size.
+: Parturition was observed, but all newborns died from the maternal cannibalism or other unknown reasons within several days after birth.
-: No parturition was observed.

Table 2-4. Body weight (g) of adult shrews from different geographical areas.

Origin	Male	Female	Reference
Taramajima Is., Japan (L)	43.5	29.0	Ishikawa *et al.*, 1989
Madagascar (L)	47.7	29.1	Hasler *et al.*, 1977
Guam (L)	44.0	26.0	Hasler *et al.*, 1977
Malaya (W)	55	45	Harrison, 1955
Nagasaki, Japan (L)	60.1	40.3	Iseki, 1978
Okinawa Is., Japan (L)	50.5	33.8	Shigehara, 1980
Tokunoshima Is., Japan (W)	61.7	33.3	Namikawa *et al.*, 1985
Sri Lanka (L)	72.9	49.6	Ishikawa *et al.*, 1989
Sri Lanka (W)	81.9	60.0	Tsubota *et al.*, 1986
West Bengal (W)	177 (Max)	103 (Max)	Louch *et al.*, 1966
Bangladesh (W)	147.3	81.7	Present data

Max: Maximum body weight.
L: Laboratory-bred shrews.
W: Wild-caught shrews.

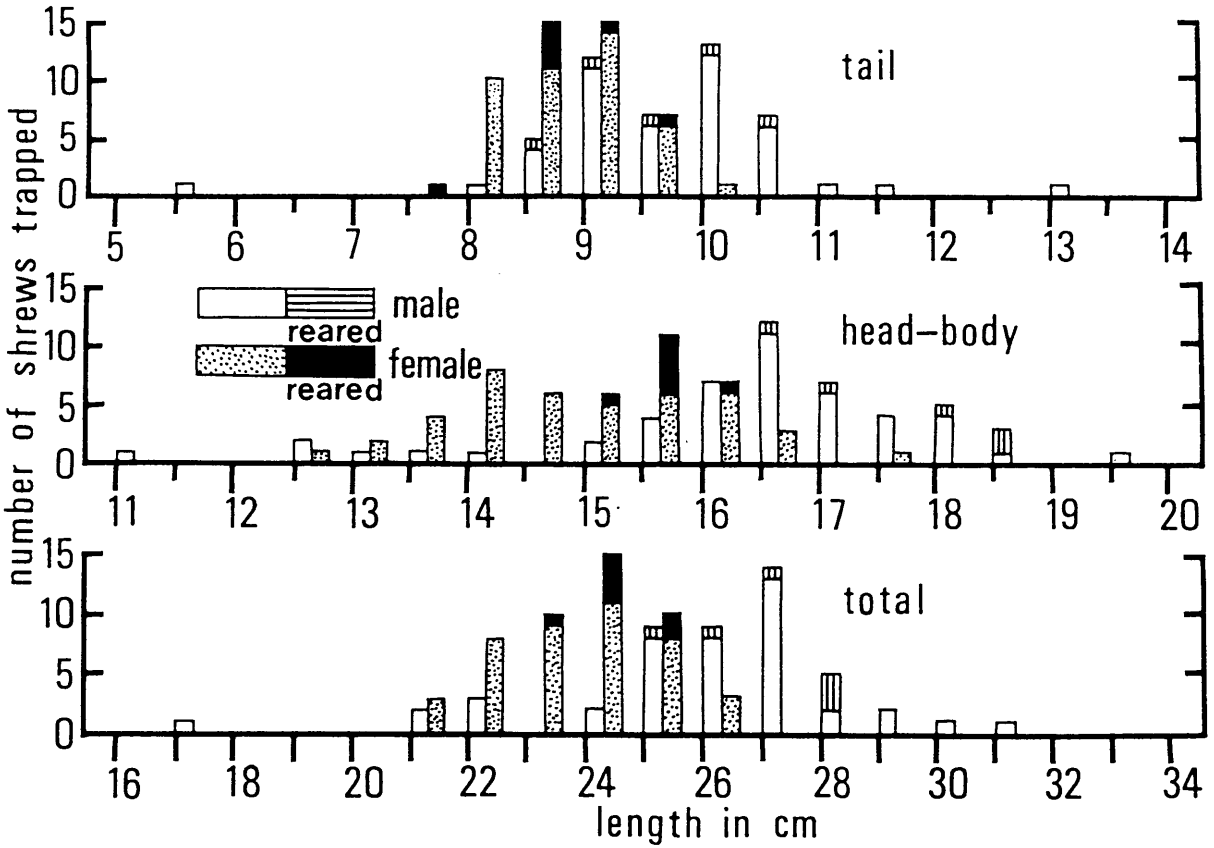

Fig. 2-1. Distribution of tail, head-body and total lengths of the wild shrews trapped in Mymensingh, Bangladesh. The darker parts of the columns indicate animals measured after being reared for more than 100 days in the laboratory. The females reared were not pregnant when they were measured. The number of shrews examined were 49 males (5 reared) and 49 females (7 reared).

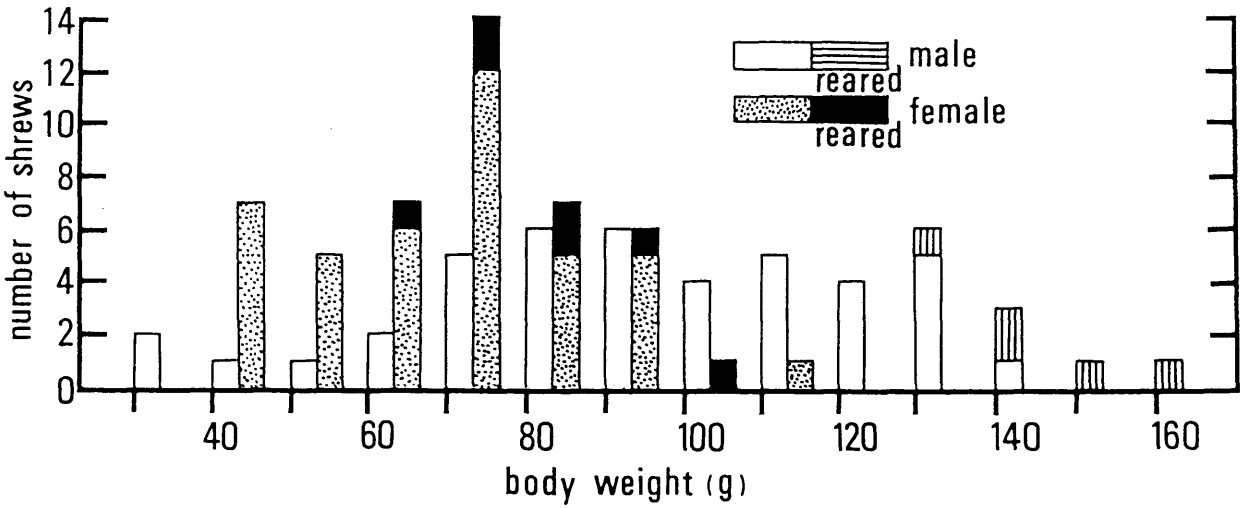

Fig. 2-2. Body weight distribution of the wild shrews trapped in Mymensingh, Bangladesh. The symbols in the figure are explained in Fig. 2-1. In this figure, two males and one females, both of which were measured roughly, were excluded from the 98 shrews shown in Fig. 2-1.

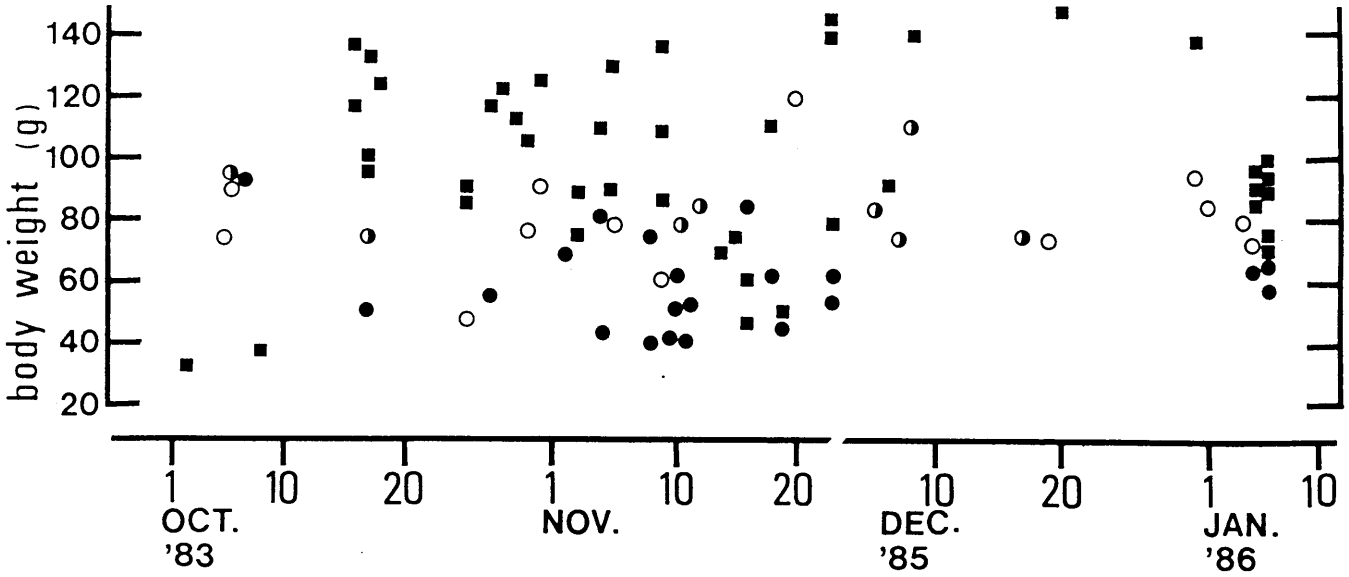

Fig. 2-3. Reproductive activities of the wild shrews trapped in Mymensingh, Bangladesh, from October to January. The solid squares show males. Circles indicate females (open circle: pregnant; half-closed circle: lactating and non-pregnant; and solid circle: non-lactating and non-pregnant). The abscissa expresses the date when the shrews were dissected for examination.

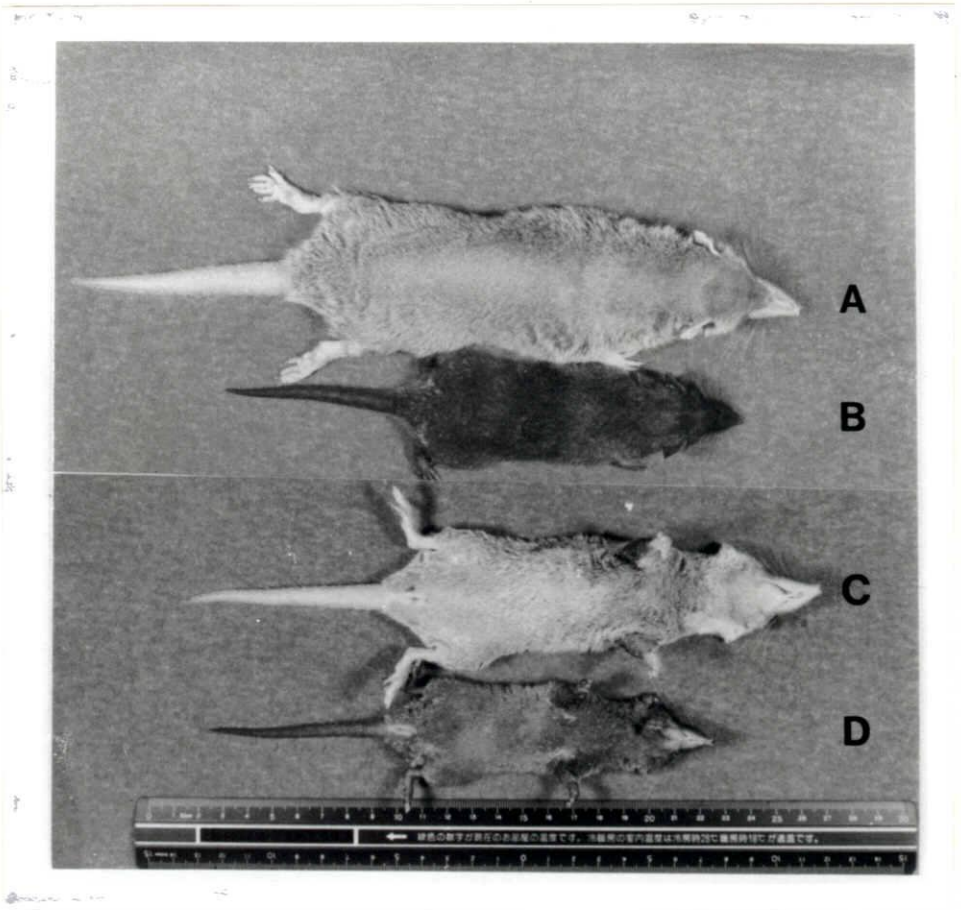

Fig. 2-4. External appearance of adult shrews from two different laboratory strains. *A* shows dorsal view of a male of the BAN strain originating from Mymensingh, Bangladesh. *B* shows dorsal view of a male of the NAG strain from Nagasaki, Japan. *C* and *D* show ventral views of females of BAN and NAG strains, respectively. Three pairs of nipples are seen on the inguinal parts of the females. BAN shrews are markedly bigger than NAG shrews in body size and exhibit a light gray coat, while NAG shrews of both sexes exhibit a dark gray coat. The belly coat is usually lighter than the dorsal coat in both strains. The ruler is 30 cm.

Chapter III

Chromosomes in the BAN strain

INTRODUCTION

The musk shrew shows extreme geographical variation in diploid chromosome number (2N). Shrews captured in Japan (Andō *et al.*, 1980; Yosida, 1982), southern Vietnam (Duncan *et al.*, 1970), Indonesia (Yosida, 1982), and northern India (Manna and Talukdar, 1967; Rao *et al.*, 1970) all had 2N=40. On the other hand, various chromosome numbers from 2N=35 to 2N=40 were reported from Malaysia by Yong (1971, 1972, and 1974) and Sam *et al.* (1979). Yosida (1982) recorded 2N=30 or 2N=32 in southern India and 2N=32 on the east coast of Sri Lanka.

However chromosomal data for Bangladesh shrews have not been published so far. As mentioned in Chapter II, I have succeeded in developing the BAN strain derived from the Bangladesh wild shrews, which are characterized by their unique genetic properties with reference to body size, mtDNA, and *Amy-1* locus. This thus leads to the greatest interest in acquiring the chromosomal information about the BAN strain.

In the present chapter, using the conventional Giemsa staining method, I describe the chromosomal characteristics of the BAN shrews in comparison with the other shrew strains.

MATERIALS AND METHODS

Shrew strains used were BAN derived from Bangladesh (Chapter II) and, for comparison, NAG from Nagasaki, Japan (Oda and Kondo, 1976), OKI from Okinawa Island, Japan (Tsubota and Namikawa, 1988), and SRI from the west coast of Sri Lanka (Ishikawa *et al.*, 1989).

Shrew chromosomes were observed in bone marrow cells from the femur using the conventional Giemsa staining and air-dry methods. The shrew was injected with 0.1 ml per 10 g body weight of 0.1% colchicine, and the bone marrow cells were flushed out 15 to 20 minutes later. The chromosomes observed were classified in accordance with the definitions provided by Levan *et al.* (1964). Sex chromosomes were identified after homologous autosomes were paired.

RESULTS

Chromosome Number

The diploid chromosome number for the BAN strain was determined to be 2N=40, which was quite identical with those of NAG and OKI strains. On the other hand, SRI shrews were found to possess 2N=30. These findings are summarized in Table 3-1.

Karyotype

Figure 3-1 shows the 2N=40 karyotype for the BAN

strain. The autosomes were classified into three groups: 4 pairs of metacentric chromosomes, one pair of submetacentric chromosomes, and 14 pairs of acrocentric chromosomes. The 4 pairs of metacentric chromosomes could be easily distinguished from one another in length, and the largest metacentric chromosomes were comparable to the largest acrocentric chromosomes in length. The submetacentric pair could be clearly identified in the complement by its unique morphology and had a secondary constitution at the distal region of the long arm. The acrocentric pairs varied gradually in length, so it was difficult to identify the homologous pairs among them (Fig. 3-1). These karyotypic characteristics of the BAN strain were not clearly different from those of NAG and OKI strains (Table 3-1).

All the three strain shrews with 2N=40 had a total of 48 autosome arms (Table 3-1).

In contrast to the 2N=40 karyotype, the autosomes of the SRI shrews with 2N=30 were composed of 9 pairs of metacentric chromosomes, one pair of submetacentric chromosomes, and 4 pairs of acrocentric chromosomes (Table 3-1). Furthermore the metacentric chromosomes could be distinctly subdivided into two groups: 5 pairs of large metacentric chromosomes and 4 pairs of small metacentric chromosomes (Fig. 3-1). The large meta-centric chromosomes, which were never found in the

2N=40 karyotype, are believed to have been formed *via* Robertsonian fusions among 10 pairs of large acrocentric chromosomes seen in the 2N=40 karyotype. This is because 1) 4 pairs of small metacentric chromosomes, one pair of submetacentric chromosomes, and 4 pairs of small acrocentric chromosomes in the SRI shrews matched those of the 2N=40 shrew chromosomes in length and morphology (Fig. 3-1); 2) the number of metacentrics in the SRI shrews increased by 5 pairs to 9 pairs, in contrast to all the 2N=40 strain shrews, while the number of acrocentrics decreased by 10 pairs to 4 pairs; and 3) the SRI shrews had the same total of 48 autosome arms as all the 2N=40 strain shrews (Table 3-1).

The X chromosomes of BAN, NAG, OKI, and SRI strains were large metacentrics. However OKI shrews seemed to have another type of X chromosome, submetacentric. On the contrary, the Y chromosome showed considerable differences in both morphology and length among the strains: a submetacentric, being much longer than the long arm of the X chromosome, for the BAN strain; a metacentric, nearly as long as the X long arm, for the NAG and OKI strains; and a submetacentric, nearly as long as the X long arm, for the SRI strain (Table 3-1 and Fig. 3-1).

DISCUSSION

The results of chromosome analysis in this study indicate that the shrew strains can be divided into two types on the basis of chromosome number: the 2N=30 type and the 2N=40 type (Table 3-1), representing the smallest and largest numbers of chromosomes reported in this species so far. The 2N= 30 type is only found in the SRI strain originating from a wild population on the west coast of Sri Lanka. However, the wild shrews caught on the east coast of Sri Lanka have been reported to have 2N=32 (Yosida, 1982). These facts provide the evidence of chromosomal polymorphism within the wild shrew population in Sri Lanka. On the other hand, the 2N=40 type is composed of the BAN strain originating from wild population of East Bengal in Bangladesh and the NAG and OKI strains from Japan. This chromosome number closely matches the numbers found in wild shrews caught in West Bengal (Manna and Talukdar, 1967) and Japan (Andō *et al.*, 1980; Obara and Miyai, 1981), these places being nearest the areas where the original breeding stocks of BAN and OKI shrews were captured.

The shrew strains examined in the present study differ greatly in adult body weight. As shown in Table 2-4 of Chapter II, the BAN shrew has more than 2 times higher body weight than the NAG and OKI shrews, whereas the SRI shrew has a body weight intermediate between the BAN and Japanese strains. There are two reports

that the BAN shrew is genetically differentiated from Japanese and SRI shrews both at the nuclear level (Tsubota and Namikawa, 1988) and mtDNA level (Yamagata *et al.*, 1987). In spite of these unique genetic characteristics of the large BAN shrew, the chromosome number of this shrew was 2N=40, *i.e.* exactly the same as in the small Japanese shrews (Table 3-1). No clear differences were demonstrated among the karyotypes of the BAN and Japanese shrews with the exception of the sex chromosomes (Table 3-1 and Fig. 3-1). Thus I believe that the 2N=40 karyotype is the standard type for this species, and this conclusion is in good agreement with the statements of Yosida (1982).

SUMMARY

The chromosomal characteristics in the BAN strain of the shrew were studied in comparison with NAG and OKI strains from Japan and SRI strain from Sri Lanka, using the conventional Giemsa staining method. The chromosome number of the BAN shrew was 2N=40, being exactly the same as in NAG and OKI shrews, whereas that of the SRI shrew was 2N=30. With the exception of the sex chromosomes, no clear differences were demonstrated among the 2N=40 karyotypes of the BAN and Japanese strains. The autosome complement was composed of 4 pairs of metacentric chromosomes, one pair of submetacentric chromosomes, and 14 pairs of acrocentric chro-

mosomes. The X chromosomes of the BAN, Japanese, and SRI strains were all large metacentrics. By contrast, the Y chromosome was a large submetacentric for the BAN strain, a small metacentric for the Japanese strains, and a small submetacentric for the SRI strain.

Table 3-1. Comparison of the karyotypes between the BAN strain and three other strains (NAG, OKI, and SRI) of the shrews.

Strain	No. of shrews examined		2N[a]	Autosomes[b] (No. of types)			Sex chromosomes		Total no. of autosome arms
	Male	Female		M	SM	A	X	Y	
BAN	2	1	40	8	2	28	M	SM	48
NAG	1	1	40	8	2	28	M	M	48
OKI	2	1	40	8	2	28	M-SM	M	48
SRI	1	1	30	18	2	8	M	SM	48

a: Diploid chromosome number.
b: M-metacentric, SM-submetacentric, and A-acrocentric chromosome.

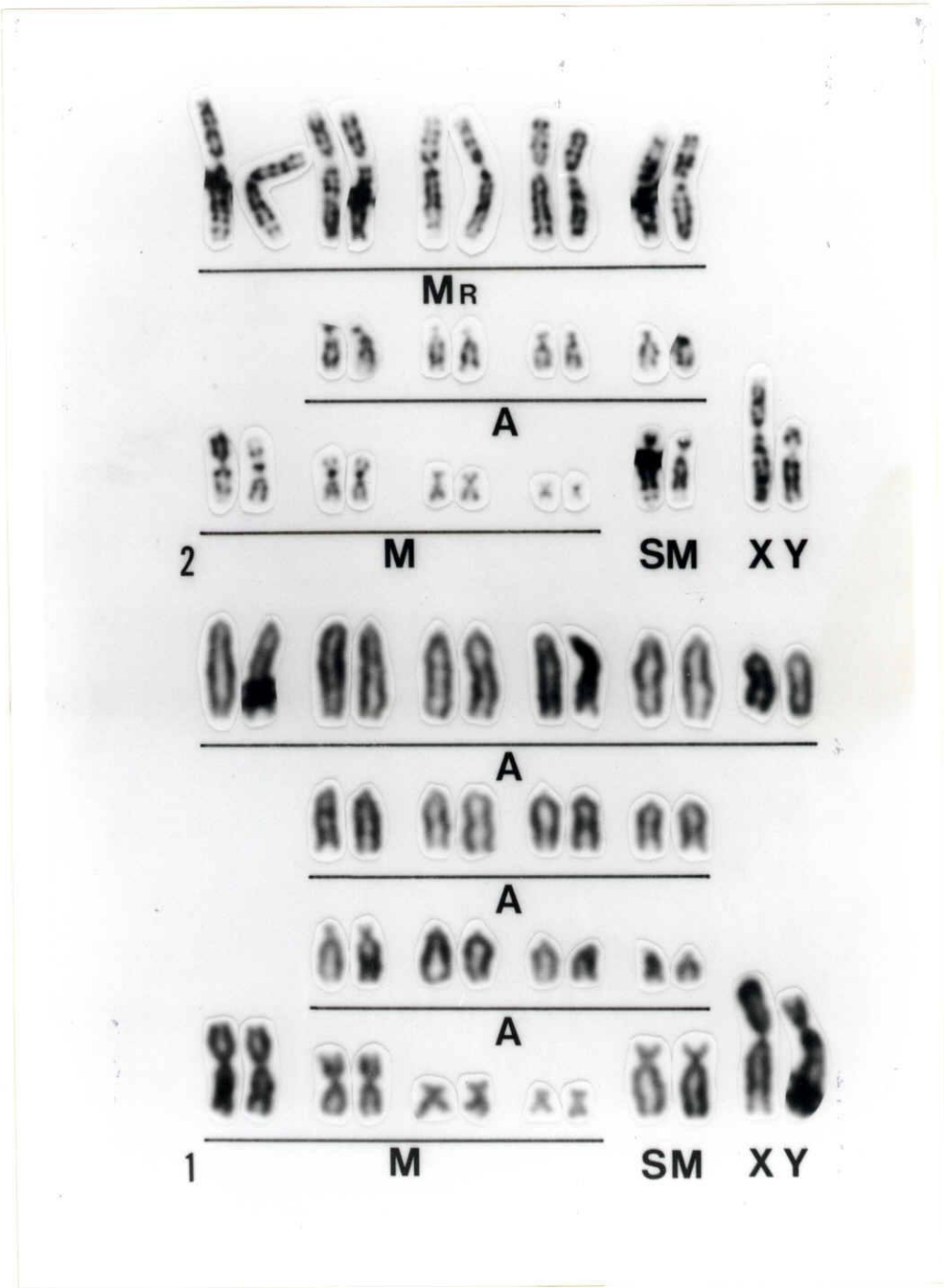

Fig. 3-1. Karyotypes of BAN (2N=40) and SRI (2N=30) shrews. 1): A BAN male with a large metacentric X chromosome and a large submetacentric Y chromosome; 2) A SRI male with a large metacentric X chromosome and a small submetacentric Y chromosome. M_R: Large metacentric chromosomes believed to have been formed by Robertsonian fusions (see text for details), M: Metacentric, SM: Submetacentric, A: Acrocentric chromosomes.

Chapter IV.

Reciprocal crosses between BAN (large size) and NAG (small size derived from Nagasaki, Japan) strains: influence of body weight difference between sexes on mating success

INTRODUCTION

In Chapter III, it was revealed that the BAN strain from Bangladesh and the NAG strain from Nagasaki, Japan have the same 40 chromosomes in diploid, and no clear differences in their karyotypes are observed with the exception of the Y chromosomes. However, as shown in Chapter II, the BAN shrew is more than 2 times heavier than the NAG shrew. Moreover the BAN and NAG shrews are genetically differentiated from each other to the level which can be compared to the mice-intersubspecific differences estimated by the mtDNA analysis (Yamagata *et al.*, 1987). I thus attempted to perform mating tests between the two extreme strains. This provided a very surprising result that F_1 hybrids between the small NAG females and the large BAN males were obtained, but no reciprocal F_1 hybrids were produced from a cross of the large BAN females to the small NAG males.

In the present chapter, I describe the underlying causes of the difference in mating success between reciprocal crosses of the large BAN shrews and the

small NAG shrews. I also examine the fertility and reproductive performance of the F_1 hybrids produced from a cross between the NAG females and the BAN males.

MATERIALS AND METHODS

The histories of BAN and NAG strains of the shrews were previously described in Chapter II and by Oda and Kondo (1976), respectively. BAN shrews used were 110 individuals from first to sixth generation after capture; NAG shrews were 78 individuals from second to eighth generation after the strain was introduced into my laboratory in 1983 from the Research Institute of Environmental Medicine, Nagoya University, Japan. Husbandry conditions for the shrews were described elsewhere (Chapter II).

Eighteen BAN and 19 NAG shrews were crossed for 2 days using the no-choice method (Coyne and Orr, 1989), in which a female of one strain was confined with a male of another strain. The 4 BAN and the 5 NAG shrews employed in this cross were cross-fostered within 36 h after birth between the strains; the remainders were non-fostered. When F_1 hybrids were obtained, they were crossed *inter se*, or reciprocally backcrossed to the two parental strains to examine the F_1 fertility. All the crosses were conducted for 2 days in single-pairs. The ages of shrews at crossing ranged from 4 to 14 months, during which the shrews were completely in

reproductive activities (Furumura *et al.*, 1985). Mating behaviors were observed twice a day for about 15 minutes. Body weight of a pair was measured to the nearest 0.01 g before crossing.

Fertility was judged from mating success, the criteria for which were pregnant females and the production of young. The pregnancy of females was examined by abdominal palpation under ether anesthesia at about 16 days after the separation of pairs. The day of birth was designated day zero after birth. Litter size and sex ratio were determined at 5 days of age, because I have experienced that the handling of pups during the first 5 days after birth induces maternal cannibalism very frequently. Percent survival was defined as the proportion of total number of pups weaned to total number of pups observed at 5 days. After weaning (20 days of age), young shrews were placed in individual cages.

Statistical comparisons were performed using χ^2-test or Fisher's exact probability test for mating success, pup survival, and sex ratio, and t-test or Cochran-Cox test for body weight and litter size.

RESULTS

The results of mating tests between large BAN and small NAG shrews are shown in Table 4-1. Neither successful mating behaviors nor pregnant females

could be observed in a total of 16 mating trials between 12 females of the large BAN shrews and 11 males of the small NAG shrews, 7 trials of which were conducted between cross- or non-fostered BAN females and cross-fostered NAG males. I observed following unsuccessful mating behaviors in the trials: BAN females were highly aggressive toward NAG males and shrieked at them; the female aggressive fighting behaviors occurred repeatedly whenever the males tried to approach the females, so the females usually stayed in nest boxes in cages and the partners were kept out throughout the mating trials. Two NAG males died from attacks by BAN females or unknown factors during the trials.

Conversely, 8 female and 8 male F_1 hybrids were produced from 6 of the 11 mating trials between 8 females of the small NAG shrews and 6 males of the large BAN shrews. Of the F_1 hybrids obtained, 5 females and 3 males were produced from 3 of the 7 trials between cross- or non-fostered NAG females and cross-fostered BAN males (Table 4-1). For the successful mating trials, aggressive behaviors were seen for the first short period when the NAG females were paired with the BAN males, but the pairs spent much of their pairing time in nest boxes together. For the unsuccessful mating trials, however, the NAG females behaved aggressively like the BAN females

described above, but there were no BAN males which died from attacks by the NAG females. Both the successful and unsuccessful mating behaviors are generally observed within the control crosses of two parental strains. One NAG female known to have given birth previously to one litter of hybrids died during the subsequent pregnancy. The litter size was 4 at autopsy. All F_1 hybrids obtained were consequently composed of only first litters that NAG females were delivered. Although one NAG female died during lactation (at 15 days after parturition), the offspring successfully grew to adulthood. These maternal deaths in the small NAG parents may result from the large size of hybrid fetuses and pups, respectively, leading to the mothers' difficult parturition and exhaustive supply with large volumes of milk.

The reproductive data for various mating combinations using BAN and NAG strains and their hybrids are summarized in Table 4-2. The F_1 hybrids obtained were fully fertile in both sexes, and had high reproductive ability. In crosses of F_1 females to any males and the cross of NAG females to F_1 males, the rates of both pregnant females and parturitions were significantly higher than those of the two parental strains. Litter size of F_1 females mated with F_1 males was significantly larger than that of NAG females mated with NAG males, and the range leaned toward larger

litter sizes. The cross between NAG females and BAN males did not differ significantly from the control crosses of BAN and NAG strains in litter size. This infers that the loss of fertilized eggs or fetuses due to immunological disparity between the two strains, as suggested in the *Peromyscus* species cross (Dawson, 1965; Dawson *et al.*, 1982), may be little. Sex ratios observed in all crosses were not significantly different from 1:1. There were no significant differences between any crosses in percent survival (Table 4-2). All young hybrids successfully reared to weaning age normally grew to adulthood, except 3 young females which died from unknown factors during the growth. All the hybrids appeared neither external nor behavioral abnormalities.

Table 4-3 presents details of body weight for sexes paired, and the influence on mating success is illustrated in Fig. 4-1. Body weights differed greatly between mating pairs in various types of crosses using F_1, F_2, and their parental shrews. Whenever females significantly exceeded males in body weight, as shown in the cross of BAN females to NAG males, the pairs did not succeed in mating at all. Conversely, many pairs could succeed in mating on condition that females did not significantly surpass males in body weight. The rate of successful matings was not significantly correlated with the difference in body weight between

sexes paired.

DISCUSSION

This is the first report on mating tests between different-sized shrews. I observed that mating success differed completely between reciprocal crosses of the large BAN shrews and the small NAG shrews. For the underlying causes, I presume two aspects: divergence in mating behaviors between the strains; influence of the body weight difference between sexes paired on mating success.

First, Hasler *et al*. (1977) reported that musk shrews from Guam and Madagascar differed significantly from each other in mating behaviors. The cross-fostering procedure is well known to affect sexual and social behaviors in animals (Huck and Banks, 1980; McGuire, 1988; McGuire and Novak, 1987). I thus cross-fostered reciprocally between BAN and NAG shrews to reduce the divergent effects of mating behaviors on crosses between the strains. However, the influence of cross-fostering on mating success between the strains was not important, because 1) in the cross of BAN females to NAG males if the differences of mating behaviors were a critical factor, then some pregnant BAN females would be obtained by means of the cross-fostered shrew cross, but I could not in fact find such females at all; 2) in the cross between NAG females and

BAN males, the percent of parturitions (75.0%: 3/4) in the non-fostered shrew cross was rather higher than that (42.9%: 3/7) of the cross-fostered shrew cross (Table 4-1). This suggests that the divergent effects of mating behaviors on the interstrain crosses may be minimal.

Second, my data demonstrated that successful matings occurred in a condition that females were nearly as heavy as their counterparts, or lighter (Fig. 4-1). In the present mating tests, the case that females were significantly heavier than males was unfortunately only the cross between the BAN females and the NAG males (Table 4-3). However, my preliminary mating tests between BAN strain and OKI strain derived from Okinawa Island, Japan, or TKU strain from Tokunoshima Island, Japan, indicated quite the same tendency as the present study. Both the Japanese strains have almost the same body weight as the NAG strain I used in this study (Fig. 4-2). Twelve female and 6 male F_1 hybrids were produced from 5 of 7 mating trials between 7 small OKI females and a large BAN male, while one female and one male F_1 hybrids were obtained by one trial between a small TKU female and a large BAN male. The F_1 hybrids obtained by both the crosses were fully fertile in both sexes. In contrast, no F_1 hybrids could be obtained by both the reciprocal crosses: 2 trials between 2 large BAN females and a

small OKI male, and 4 trials between 2 large BAN females and 4 small TKU males. In the crossing of musk shrews from Guam and Madagascar, both of which had almost the same body weight in each sex, the reciprocal F_1 hybrids were produced (Hasler *et al.*, 1977).

Moreover, the musk shrew, an induced ovulatory animal (Dryden, 1969), shows extreme sexual dimorphism in adult body weight: males are constantly about 1.7 times heavier than females (Fig. 4-2). As a rule, the onset of copulatory activity in the shrew is primarily under female control (Rissman, 1987; Rissman and Bronson, 1987). When females first encounter males, they are immediately and highly aggressive toward the males. In successful matings, females reduce their aggressive fighting behaviors, and become receptive, after that mounting copulations occur. Detailed mating behaviors are described by Hasler *et al.* (1977) and Rissman (1987). In unsuccessful matings, males and females become more aggressive, or males run away from areas females occupy. Male aggressiveness appears to play an important role in mating success, as Dryden (1969) suggested. My result on the cross between the large BAN females and the small NAG males is a typical example of the unsuccessful matings (Table 4-1). I conceive that when females much exceed males in body weight, female aggressiveness is greatly reinforced. I may therefore infer that the reinforced aggressive

fighting behaviors of the large BAN females never allowed the small NAG males to mount and copulate.

From the above facts, I conclude that differences in body weight between females and males significantly affect mating success in the induced ovulatory shrews.

In addition to the body size difference, BAN and NAG shrews possess different genetic characteristics. Based on the nucleotide diversity of mtDNA, the degree of genetic differentiation between the two strains is comparable to the extent which can be intersubspecific differences in *Mus musculus* (Yamagata *et al.*, 1987). The BAN shrews are covered with light-gray pelage and pale-pink skin, whereas the NAG shrews have the coat and skin both colored dark gray (Chapter II). Although the musk shrew shows extreme variation in a diploid chromosome number, ranging from 2N=30 to 2N=40 (Duncan *et al.*, 1970; Yosida, 1982; Yong, 1971), both the present strains have 2N=40 and a total of 48 autosome arms (Chapter III). However the Y chromosome is a submetacentric in the BAN strain and a metacentric in the NAG strain (Chapter III).

In the present chapter, I revealed that mating success was closely associated with body weight. This gives me the inference that differences in mating preference exist between reciprocal crosses of the BAN and NAG strains. Lande (1988) suggests that such a mating preference is a reproductive isolation barrier

between populations of animals. I therefore believe that the BAN and NAG strains originating from different geographical races have evolved a partial premating isolation mechanism likely caused by the difference in body weight between them, because viable and fertile hybrids are produced from a cross between the small NAG females and the large BAN males (Table 4-2) despite their genetic differences described above. The systematic observations of sexual behaviors in the two strains will provide the definitive evidence for this isolation mechanism.

SUMMARY

I conducted mating experiments between BAN (large size) and NAG (small size) strains of the shrews. In the 16 mating trials between 12 BAN females (mean body weight of 87.9 g) and 11 NAG males (52.3 g), highly aggressive fighting behaviors of the BAN females toward the NAG males were observed. The BAN females stayed in nest boxes in cages, whereas the NAG males were out during the mating trials. No pregnant females were found in the trials. Their pregnancies were diagnosed by palpation on the day about 16 days after separation of the pairs. On the contrary, in 6 of the 11 trials between 8 NAG females (34.2 g) and 6 BAN males (145.9 g), the viable and fertile F_1 hybrids of 8 females and 8 males were produced. The F_1, subsequent F_2 and

backcross progenies appeared neither external nor behavioral abnormalities. Various types of crosses using the F_1, F_2, and the two parental shrews showed that mating success was in a condition that females were nearly as heavy as males, or lighter. Although body weights of musk shrews from different geographical areas were reported to vary from 43.5 to 147.3 g in males and from 26.0 to 82.0 g in females, males in respective localities were constantly about 1.7 times heavier than the corresponding females. These results therefore suggest that the difference in body weight between sexes paired greatly affect mating success in the cross between the strains of induced ovulatory shrews.

Table 4-1. Mating experiments between BAN and NAG strains of the shrews.

Cross Female X Male	No. of animals: Female	No. of animals: Male	No. of different mating Pairs	Total no. of mating trials	Proportion of pregnant females (%)	Proportion of parturitions (%)	Total no. of offspring obtained: Female	Total no. of offspring obtained: Male
BAN^C X NAG^C	2	2	2	4	0	0	0	0
BAN^N X NAG^C	3	2[a]	3	3	0	0	0	0
BAN^N X NAG^N	7	8	9	9	0	0	0	0
Total	12	11	14	16	0	0	0	0
NAG^C X BAN^C	2	2	2	2	100.0	100.0	4	3
NAG^N X BAN^C	2	1[b]	2	5	20.0	20.0	1	0
NAG^N X BAN^N	4	4	4	4	100.0	75.0	3	5
Total	8	6	8	11	63.6	54.5	8	8

C: Cross-fostered shrews.
N: Non-fostered shrews.
a: One of the two males was used in the BAN^C X NAG^C cross.
b: The same male was used in the NAG^C X BAN^C cross.

Table 4-2. Fertility and reproductive performance in crosses between BAN and NAG strains of the shrews.

Cross	No. of animals		No. of different mating pairs	Total no. of mating trials	Proportion of pregnant females (%)	Proportion of parturitions (%)
Female X Male	Female	Male				
BAN X BAN	27	45	124	128	15.6^{b}	14.1^{b}
NAG X NAG	24	22	65	70	31.4^{ce}	25.7^{c}
BAN X NAG	12	11	14	16	0^{b}	0^{b}
NAG X BAN	8	6	8	11	63.6^{ac}	54.5^{ac}
F_1 X F_1	7	6	16	22	77.3^{a}	77.3^{a}
F_1 X BAN	7	6	10	10	70.0^{ad}	70.0^{ad}
F_1 X NAG	4	3	5	6	100.0^{a}	100.0^{a}
BAN X F_1	18	8	26	32	25.0^{be}	25.0^{bc}
NAG X F_1	11	6	12	12	66.7^{ad}	66.7^{ad}
F_2 X F_2	25	17	51	53	39.6^{cde}	34.0^{cd}

(to be extended)

Table 4-2. (Extended).

Cross Female X Male	Litter size at 5 days n	$\bar{X}\pm SD$	Range	Sex ratio at 5 days Female : Male	% Survival at weaning#
BAN X BAN	17	3.6 ± 1.1^{ab}	2 - 5	28 : 31	96.6 (57/59)
NAG X NAG	17	2.8 ± 1.1^{cd}	1 - 5	20 : 28	91.7 (44/48)
BAN X NAG					
NAG X BAN	6	2.7 ± 1.0^{bcd}	1 - 4	8 : 8	93.8 (15/16)
F_1 X F_1	13	4.3 ± 1.0^{a}	3 - 6	35 : 21	96.4 (54/56)
F_1 X BAN	6	3.7 ± 0.5^{ac}	3 - 4	11 : 11	100.0 (22/22)
F_1 X NAG	5	3.6 ± 1.1^{ad}	2 - 4	11 : 7	100.0 (18/18)
BAN X F_1	7	2.3 ± 1.1^{bd}	1 - 4	9 : 7	87.5 (14/16)
NAG X F_1	8	2.7 ± 0.5^{d}	2 - 3	11 : 10	100.0 (21/21)
F_2 X F_2	16	2.9 ± 1.4^{bcd}	1 - 5	23 : 24	93.6 (44/47)

n: Number of cases observed.
a-d: Values with the same letters are not significantly different at $P<0.05$.
#: Proportion of total number of pups weaned (20 days of age) to total number of pups observed at 5 days is indicated in parentheses.

Table 4-3. Body-weight (g) comparisons in mating pairs employed in crosses between BAN and NAG strains of the shrews.

Cross			Female		Male		Male/Female[a]	
Female	X	Male	*n*	$\bar{X}\pm SD$	*n*	$\bar{X}\pm SD$	*n*	$\bar{X}\pm SD$
NAG	X	BAN	7[b]	34.2±2.8	5[b]	145.9±16.8***	7[b]	4.2±0.5
F_1[c]	X	BAN	7	51.7±4.9	6	128.0±15.3***	10	2.6±0.3
NAG	X	F_1	10[b]	32.9±1.7	6	83.4±14.1***	11[b]	2.5±0.4
F_2	X	F_2	25	55.1±6.1	17	103.9±14.2***	51	1.9±0.3
F_1	X	F_1	7	51.7±4.9	6	90.4±13.2***	16	1.8±0.3
BAN	X	BAN[d]	28	82.0	20	135.3***		1.7
NAG	X	NAG[d]	16	34.2	27	52.9***		1.5
BAN	X	F_1	18	80.2±8.3	8	86.0±13.9	26	1.1±0.2
F_1	X	NAG	4	52.1±3.7	3	53.9± 7.2	5	1.0±0.1
BAN	X	NAG	11[b]	87.9±9.2	9[b]	52.3± 6.6***	12[b]	0.6±0.1

n: Number of shrews weighed.
a: Weight ratio in different female-male combinations.
b: Animals not weighed are excluded (see Table 4-2).
c: F_1 hybrids produced from the NAG X BAN cross.
d: Weights quoted from Chapter III.
***: Significantly different from females at $P<0.001$.

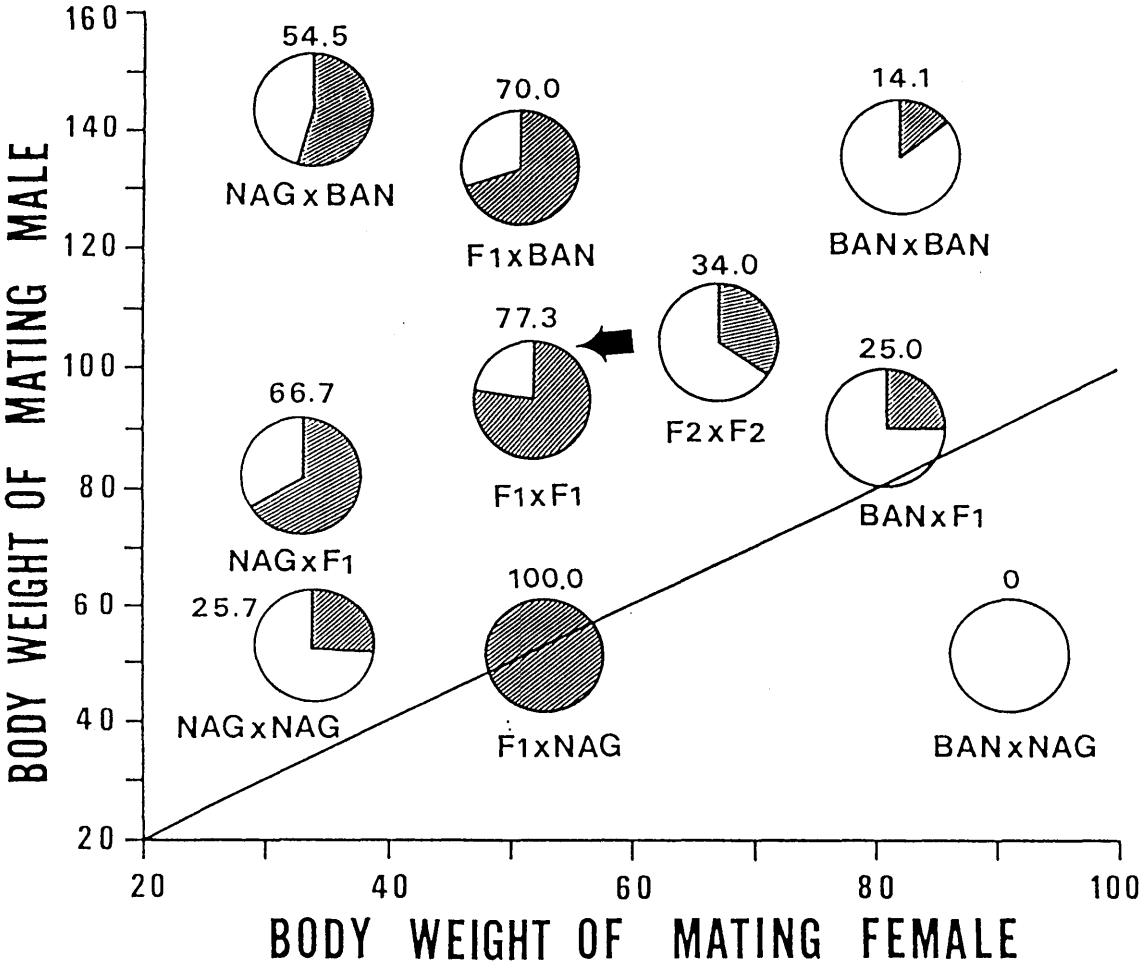

Fig. 4-1. Body-weight (g) influence on mating success in crosses between BAN and NAG strains of the shrews. Numbers indicate the percent of parturitions (see Table 4-2), depicted by circle graphs. The center of circles represents the average body weight of mating pairs in respective crossing groups (see Table 4-3 for details). The arrow shows the right position at which pairs in the F_2 X F_2 cross occupy. Solid line indicates the points where sexes are equal in body weight.

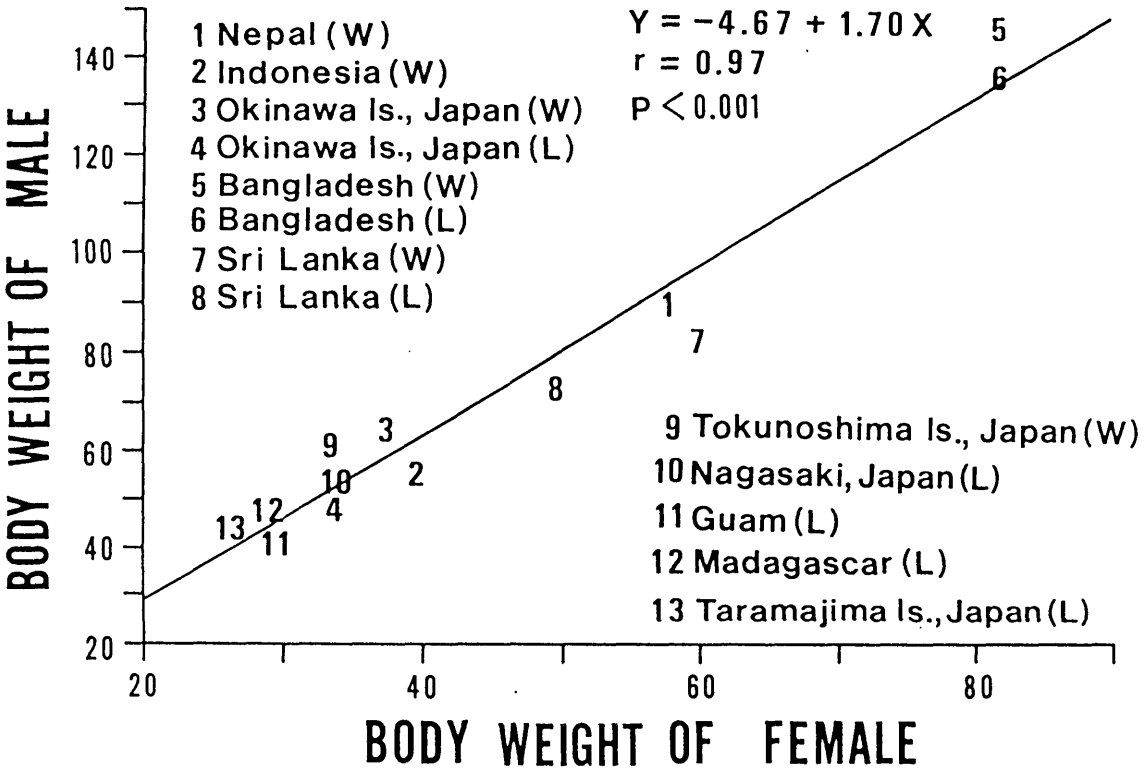

Fig. 4-2. Sexual dimorphism in adult body weight (g) of the shrews from different geographical areas. (W) and (L) indicate wild-caught and laboratory-bred shrews, respectively. Present weight data for wild-caught shrews are as follows: 1, 89.5±10.7 g ($\bar{X}\pm SD$) in 4 males and 57.9±4.9 g in 6 females in Kathmandu, Nepal (collected in 1988-1989); 2, 55.1±5.3 g in 10 males and 39.5±5.5 g in 21 females in Bogor, Indonesia (1988-1989); 3, 64.8±6.7 g in 9 males and 37.6±7.2 g in 12 females in Okinawa Island, Japan (1987). The other data are quoted from following reports: 4, Shigehara (1980); 5, Chapter II; 6 and 10, Chapter III; 7, Tsubota *et al.* (1986); 8 and 13, Ishikawa *et al.* (1989); 9, Namikawa *et al.* (1985); 11 and 12, Hasler *et al.* (1977).

Chapter V.
Postnatal growth and development in BAN and NAG strains

INTRODUCTION

In Chapter IV, I revealed that fertile and viable F_1 shrew hybrids are obtained by crossing large BAN males with small NAG females, nevertheless no reciprocal F_1 progeny of small NAG males and large BAN females are produced and the cause is due to the difference in body weight between the sexes paired. Generally the shrew is known to show extreme geographical variation in adult body weight, ranging from 43.5 g to 147.3 g in males and from 26.0 g to 82.0 g in females (see Table 2-4 and Fig. 4-2). There are several reports on postnatal growth and development of the shrews under laboratory conditions (Dryden, 1968; Furumura *et al.*, 1984; Iseki, 1978; Naruse *et al.*, 1978; Oda and Kondo, 1976), but fewer studies have attempted to relate geographical differences in adult body size to differences in the growth process.

In the present chapter, I examine postnatal growth and development in large shrews of the BAN strain and small shrews of the NAG strain in an attempt to assess the underlying causes of the difference in adult body weight between the two strains.

MATERIALS AND METHODS

Detailed histories of BAN and NAG strains of the shrews have been described in Chapter II and by Oda and Kondo (1976), respectively. Both strains have been maintained in the laboratory as closed breeding colonies. Deliberate selection and close inbreeding have been avoided. BAN shrews examined were 68 individuals from first to third generation after capture, and NAG shrews were 80 individuals from second to fourth generation after the strain was introduced into my laboratory in 1983 from the Research Institute of Environmental Medicine, Nagoya University, Japan. Husbandry conditions for the shrews were mentioned in Chapter II.

A female was mated with a male introduced into her cage for 2 days. Gestation period was counted from the separation of the pair to birth of young. Nests of pregnant females were checked several times a day, and the day of birth was designated day zero for growth measurements. Litter size was determined at 3 or 5 days of age. Young shrews were removed from their mother at 20 days and placed in individual cages. Thirteen developmental events (see Fig. 5-1) were selected for study following Dryden (1968). Body weight of young shrews was measured (to nearest 0.01 g) in two groups of each strain from birth to 120 days of age. Animals in one group were weighed at 5-day

intervals from days 3 to 18, every 7 days to day 88, and every 10 days to day 118 and on day 120; in the other group, weight was determined at 5-day intervals from birth to day 20, then every 7 days to day 90, and at 10-day intervals to day 120. This protocol was followed to avoid cannibalism by mothers that might have resulted from more frequent handling of young. Some newborn were weighed before the first suckling.

In accordance with the procedure described by Yamagishi (1977), the growth equation, which was composed of linear and decaying exponential functions (Bertalanffy, 1960), was computed to fit the growth data of each strain. The linear function is represented by

$$W(t) = at + b$$

and the decaying exponential function is expressed by

$$W(t) = K(1 - e^{c-\lambda t})$$

where $W(t)$ is body weight (g) at age t (days); a, c, and λ are constant values; b is birth weight; K is final body weight; and e is the base of natural logarithms.

No cannibalism by mothers was observed. Data from 2 BAN and 10 NAG shrews that seemed ill or dwarfed or that died before weaning were discarded. Statistical comparisons were made using χ^2- or t-tests.

RESULTS

Gestation Period, Litter Size, and Sex Ratio

Gestation period of all shrews in BAN and NAG strains was between 28 and 32 days. Litter size ($\bar{X}\pm SD$) was 3.0±1.2 (range 1 to 5, 23 litters) in BAN strain and 2.7±1.3 (range 1 to 5 for 30 litters) in NAG strain (Table 5-1). The most frequent litter size was 2 or 3 in both strains. There was no significant difference between strains in mean litter size ($0.2<P<0.5$). Male:female ratios at 5 days of age were 33:35 in BAN strain, and 43:37 in NAG strain. In neither strain did the sex ratio differ from 1:1.

Postnatal Development

Patterns for 13 developmental events in BAN and NAG strains are shown in Fig. 5-1.

Characteristics of newborn.--Six and 15 newborn less than 3 h old were observed in BAN and NAG strains, respectively. Their skin was reddish-pink and faded to pale pink at 1 day of age. Dorsal and belly skin was sparsely covered with colorless guard hairs about 1 mm long. Facial vibrissae were about 3 mm long and colorless. Colorless guard hairs 1 to 2 mm long were thickly dispersed on the tail.

The pink iris and the black sclera were visible through sealed eyelids. The ears were closed and pinnae closely applied to the lateral parts of the head. Well-formed claws were present. The skin between the digits was fused along the proximal one-

half of the digits. The urodeal lips of the ostium urogenitoanalis were sealed but the coprodeal lips were separated. The stomach (distended with milk), the liver, and the intestine were visible through the abdominal wall. Females had three pairs of inguinal nipples at birth. There were no differences in any of these characters between BAN and NAG strains.

Skin and pelage.--When the onset of darmal pigmentation became obvious, the dorsal and belly skin were light gray in BAN strain but dark gray in NAG strain. The dorsal skin was slightly darker than the belly in both strains. The muzzle, limbs, tail, and pinnae were pigmented dark gray in NAG strain, but were not pigmented in BAN strain.

In both strains, small pieces of skin flaked off dorsal and belly parts from the head to the rump a few days before the first underfur was visible. After that, light gray fur covered the body of BAN shrews, and dark gray fur in NAG shrews. The belly fur was usually lighter gray than the dorsal. Bare bases of the nipples in females of both strains contrasted with the belly fur.

Eyes, ears, and digits.--All shrews in both strains had black irises at 3 days of age. Subsequently, a narrow slit in the eyelids was visible. Often, one eye opened first and the other opened the following day.

The ear notch formed from days 3 to 8 and the adult-like ear was formed by 8 days of age. After the ears opened, caravanning behavior (Tsuji and Ishikawa, 1984) was observed even if the eyes were still closed. Digits of the fore feet separated about 1 day earlier than those of the hind feet.

After 10 days of age, young of both strains resembled adults but were easily distinguished from adults by the slightly lighter coat color. Apart from differences in skin and coat color, developmental patterns were quite similar between sexes within a strain and between the two strains.

Postnatal Growth

Body weight.--Data on body weight from birth to 120 days of age in both strains are presented in Table 5-2. Mean body weight after the first suckling was roughly 1.4 times that before suckling in both strains. Mean body weight of newborn BAN males (before suckling) was significantly higher than for NAG males (t-test, $0.01<P<0.05$). At day zero, BAN shrews were already larger than NAG shrews of both sexes. Three-day old shrews of both sexes and strains were more than three times heavier than newborn (before suckling). At weaning (20 days of age), BAN young averaged 62.9 g in males and 46.6 g in females, compared with NAG young at 34.2 g and 28.2 g, respectively. At 120 days of age, BAN shrews weighed 135.3 g (range 112.7 to 173.2 g) in

males and 82.0 g (67.6 to 115.7 g) in females, while NAG shrews weighed 52.9 g (47.0 to 58.5 g) and 34.2 g (30.2 to 38.8 g), respectively.

Figure 5-2 shows body weight growth curves. In both strains, weight increased at a relatively constant rate for several weeks after birth, followed by a period of growth at progressively declining rates. Therefore, equations combining linear and decaying exponential functions reasonably describe growth data of the shrew strains. Body weight differed significantly between sexes at 13 days of age (t-test, $0.01<P<0.05$) in BAN strain and 15 days ($P<0.01$) in NAG strain, and these differences were amplified with increasing age.

Relative growth curves of the two strains are shown in Fig. 5-3. The relative growth curve of each strain was divided into two parts: the growth phase, from birth to the day when 95% of final body weight was attained, and a subsequently stationary phase in which growth generally ceased. In BAN strain, the growth phase was retained for 56 days in males and for 48 days in females; respective values for the NAG strain were 63 and 40 days. These values varied little within each sex-strain group.

The growth phase could be further subdivided into two portions: a linear growth phase during which growth rates were constant, and a decaying growth phase, in

which the rate of gain in body weight decreased exponentially (Fig. 5-4). In the BAN strain, linear growth was maintained for 34 days in males and 25 days in females, compared with only 15 and 10 days, respectively, in NAG males and females. By the end of the linear growth phase, BAN strain had reached 76% of final body weight in males and 69% in females, compared with values of 53% and 55%, respectively, for NAG males and females. It is clear that, for both sexes, NAG strain grew much earlier than BAN strain during the linear growth phase (Fig. 5-3).

Growth rate.--Figure 5-4 represents growth rates of the two strains. Both sexes of BAN strain retained higher growth rates than the NAG strain during the linear growth phase. Growth rates during this phase were 3.0 g/day in males and 2.1 g/day in females for BAN strain, while respective rates for NAG strain were 1.7 and 1.6 g/day.

DISCUSSION

Observed gestation periods of BAN and NAG strains were about 30 days. This value agrees closely with previous reports (Dryden, 1969; Morita, 1964). Litter size is well known to affect postnatal growth in laboratory species (Azzam *et al.*, 1984; Hanrahan and Eisen, 1974; Legates, 1972; Rahnefeld *et al.*, 1966). In the present study, there was a consistent tendency

for young shrews in litters of one to be heavier during the preweaning period than those from litters of five, but the difference was not statistically significant. Differences in postweaning body weight between animals from small and large litters were also reduced, perhaps due to compensatory growth, as discussed by Monteiro and Falconer (1966) for mice. Litter sizes did not affect the timing of developmental events. Furthermore, there was no significant difference in mean litter size in the two strains. These data suggest that the influence of litter size on postnatal growth and development is unimportant for comparison of the two strains.

The ages at which major developmental events occurred in BAN and NAG strains were almost identical to those in the musk shrew from Guam (Dryden, 1968; Dryden and Ross, 1971) and those reported for the NAG strain by Iseki (1978), Naruse *et al*. (1978), and Oda and Kondo (1976). Geographical origin or husbandry conditions thus do not appear to affect developmental timing in musk shrews.

Because pre-suckling BAN newborn were in fact fetuses, their body weight may be underestimated (see Table 5-2). However, use of fetus weight does not seriously affect the computed growth rate for young shrews of BAN strain because BAN shrews grow linearly for several weeks just after birth.

Growth of BAN and NAG strains is rapid during the early postnatal stage. Body weights of BAN and NAG strains were more than twice those of newborns by 1 or 2 days of age. Musk shrews from Guam grew to more than double their birth weight by 3 days of age (Dryden and Ross, 1971), while rats, rabbits, and cats require 5, 6, and 7 days, respectively, to double the birth weight (Bernhart, 1961). The musk shrew has a remarkably high growth rate for several days after birth. In mice, growth curves have an inflection point at about 30 days after birth; at this point, growth rate is maximal (Gall and Kyle, 1968). In contrast, the growth curves of musk shrews in this study had no inflection (Fig. 5-2) because growth was constant for several weeks after birth, and subsequently growth rates rapidly decreased (Fig. 5-4). To explain this retention of high growth rate, Dryden and Anderson (1978) suggested that musk shrews produce large volumes of milk or unusually rich milk.

The growth period of BAN and NAG shrews is nearly identical to that reported for musk shrews from Guam (Dryden, 1968) and Okinawa, Japan (Shigehara, 1980). However, the phase of linear growth was about 2.5 times longer in BAN than NAG animals of both sexes, and growth rates during this phase were higher in BAN than in NAG shrews (Fig. 5-4).

In both sexes, the difference in body weight

between these strains increased linearly and rapidly, and ultimately remained constant (Fig. 5-2). The difference at 120 days of age (= adult body weight difference; calculated from growth equations in the legend of Fig. 5-2) was 82.8 g in male and 48.2 g in female. Much of the difference between strains in adult body weight was produced between the end of the linear growth phase of BAN strain and that of NAG strain: the difference in growth over 19 days (from 15 to 34 days of age) was equivalent to 50% of the difference in adult body weight in males; growth during a 15-day period (10 to 25 days of age) accounted for 44% of the difference in body weight of females (Fig. 5-5).

From these results, I conclude that the difference in adult body weight in these strains are caused by differences both in the duration of linear growth and in the growth rate during this phase.

SUMMARY

Postnatal growth and development were studied in large BAN and small NAG shrews in the laboratory. For the BAN strain, mean body weight at 120 days of age was 135.3 g in males and 82.0 g in females, compared with 52.9 g and 34.2 g, respectively, in NAG males and females. There was no significant difference between strains in gestation period (about 30 days) or in mean

litter size. Timing of postnatal appearances of 13 characters was nearly identical in the two strains. The period from birth to the day when the shrew reached a body weight plateau could be divided into two phases: a linear growth phase followed by a period of gradually declining growth rate. The growth phase was about 60 days long in males and about 40 days in females of both strains. However, BAN strain exhibited linear growth for 34 days in males and for 25 days in females, whereas NAG males and females did so for only 15 and 10 days, respectively. Growth rates during the linear growth phase were 3.0 g/day in males and 2.1 g/day in females for BAN strain, while for NAG strain the respective rates were 1.7 and 1.6 g/day. Consequently, the difference in adult body weight between the two strains reflect both the longer duration of the linear growth phase and the higher growth rate during this phase in BAN strain.

Table 5-1. Distribution of litter sizes determined at 3 or 5 days of age in BAN and NAG strains of the shrews.

Litter size	Number of cases	
	BAN strain	NAG strain
1	2	6
2	7	9
3	7	7
4	4	5
5	3	3
$\overline{X}\pm SD$	3.0±1.2	2.7±1.3

Table 5-2. Postnatal changes in body weight (g) of BAN and NAG strains of the shrews. Age is in days. Each value indicates the mean±*SD*. Number of shrews weighed is indicated in parentheses.

	BAN strain				NAG strain			
Age	Male		Female		Male		Female	
0(B)	(4)	3.0±0.4*	(1)	2.7*	(6)	2.3±0.3	(7)	2.2±0.4
0(A)	(4)	3.6±0.6	(2)	3.7±0.4	(2)	3.2±0.1		
3	(10)	11.2±2.2	(14)	9.4±1.3	(16)	7.2±1.3	(13)	7.3±0.9
5	(9)	15.5±3.9	(14)	14.2±4.3	(11)	10.9±1.3	(7)	10.3±2.1
8	(10)	26.8±5.4	(14)	20.1±3.1	(18)	15.1±3.1	(15)	14.7±1.9
10	(11)	31.6±8.4	(14)	26.5±5.5	(16)	20.5±2.6	(9)	18.7±2.2
13	(10)	43.0±9.3	(14)	30.3±5.8	(16)	23.0±4.2	(14)	20.7±2.8
15	(11)	47.7±12.3	(14)	36.8±7.3	(9)	28.3±4.0	(6)	24.4±1.5
18	(12)	55.4±11.3	(14)	40.8±6.8	(16)	28.8±4.0	(14)	25.3±2.9
20	(11)	62.9±15.7	(14)	46.6±8.2	(11)	34.2±5.5	(9)	28.2±2.3
25	(9)	77.2±7.7	(14)	55.4±7.9	(10)	38.5±2.7	(11)	29.3±2.6
27	(13)	80.5±18.5	(14)	59.1±7.9	(11)	39.8±4.9	(9)	29.3±2.1
32	(9)	101.3±12.5	(14)	67.3±7.5	(10)	42.9±2.3	(11)	31.0±2.2
34	(13)	99.3±19.9	(14)	66.7±7.7	(10)	42.8±4.6	(8)	30.4±2.7
39	(7)	122.1±14.4	(10)	78.7±8.3	(10)	45.8±3.2	(9)	30.8±2.3
41	(13)	114.7±22.3	(14)	71.7±8.2	(5)	46.9±6.1	(7)	31.7±2.6
46	(9)	122.6±16.4	(14)	78.7±10.6	(10)	46.6±3.8	(11)	32.1±3.1
48	(13)	119.2±21.3	(14)	75.3±8.8	(10)	47.9±5.4	(8)	31.9±2.6
53	(9)	129.2±17.3	(14)	81.2±12.1	(10)	48.0±4.6	(9)	32.9±3.3
55	(13)	121.8±20.4	(14)	75.9±9.5	(10)	49.3±4.3	(7)	32.4±1.2
60	(9)	130.2±17.4	(14)	81.7±14.0	(10)	48.8±3.3	(9)	33.3±3.5
62	(10)	128.6±20.7	(12)	78.0±10.5	(10)	51.0±4.1	(8)	33.6±2.2
67	(9)	130.9±18.4	(14)	82.0±14.4	(10)	49.2±3.3	(9)	33.9±3.7
69	(13)	125.6±21.2	(14)	77.9±9.9	(11)	50.8±3.4	(8)	34.2±2.2
74	(8)	136.8±18.9	(14)	83.2±15.8	(10)	48.5±3.1	(9)	34.0±3.2
76	(10)	130.9±21.3	(12)	77.5±10.2	(11)	51.6±3.8	(7)	34.3±3.3
81	(8)	140.5±18.2	(14)	84.4±15.6	(10)	49.7±3.4	(9)	35.0±3.4

Table 5-2. (Continued).

Age	BAN strain		NAG strain	
	Male	Female	Male	Female
83	(13) 128.0±20.4	(14) 77.8±9.9	(11) 51.9±3.3	(8) 34.5±4.2
88	(8) 140.1±18.6	(14) 85.5±16.0	(10) 51.0±4.8	(9) 34.9±3.3
90	(12) 129.6±21.0	(14) 76.7±9.3	(10) 53.9±3.6	(7) 35.2±2.9
98	(8) 139.2±21.4	(14) 84.5±15.2	(10) 51.3±3.8	(9) 33.9±2.7
100	(12) 129.9±19.5	(14) 76.9±8.0	(9) 51.9±3.3	(6) 32.4±2.1
108	(8) 140.8±18.8	(14) 85.1±14.9	(10) 51.4±4.2	(9) 34.2±3.1
110	(12) 130.1±20.1	(14) 77.2±7.9	(9) 52.8±3.8	(4) 33.0±3.2
118	(8) 139.7±19.4	(14) 86.1±14.1	(10) 51.5±3.9	(9) 34.3±2.9
120	(20) 135.3±18.5	(28) 82.0±12.1	(27) 52.9±3.6	(16) 34.2±2.7

A: Newborn animals after the first suckling.
B: Newborn animals before the first suckling.
*: Weight of fetuses obtained by Caesarean operation of females estimated to be on day 30 of pregnancy.

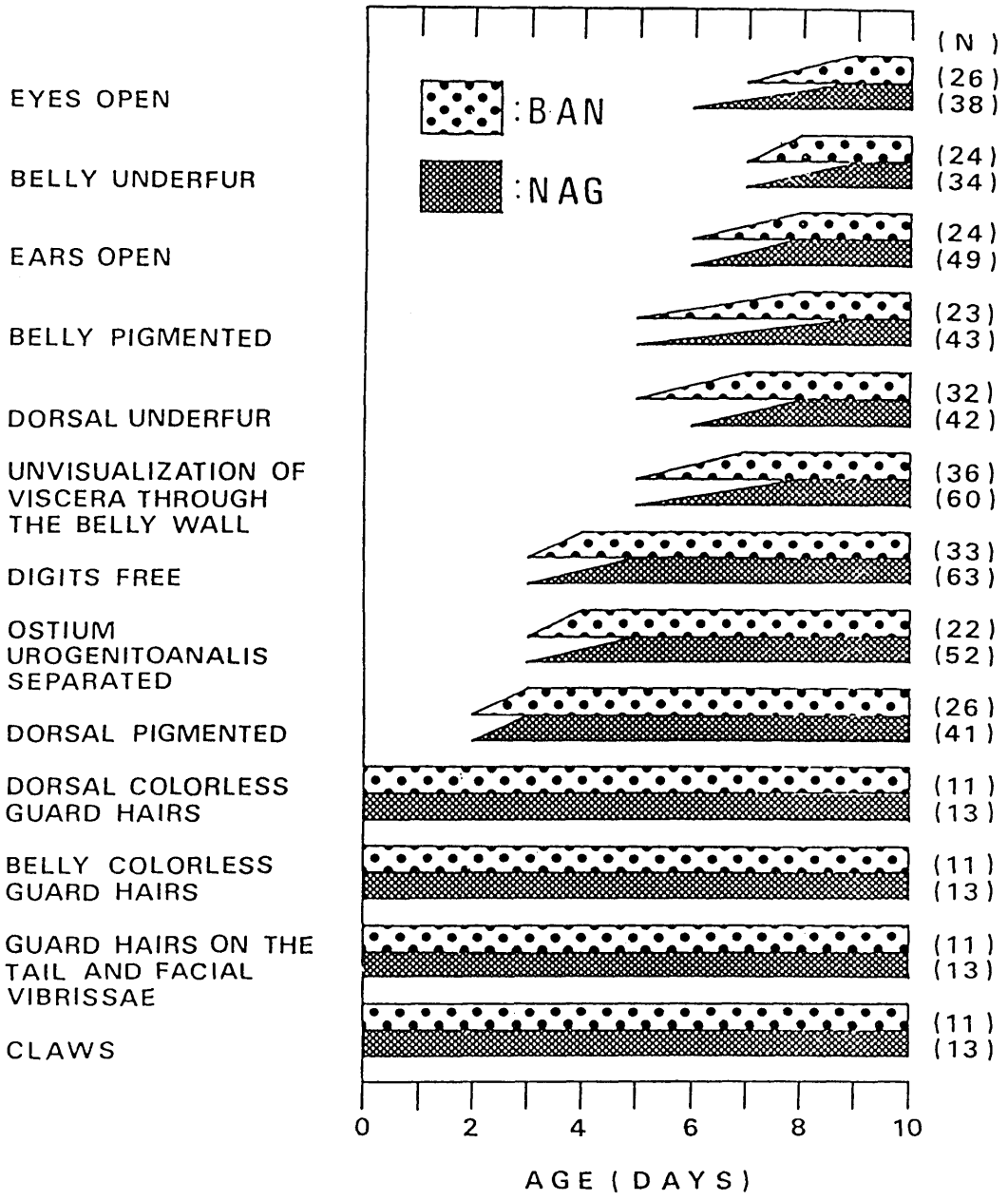

Fig. 5-1. Comparison of developmental events in the postnatal period in BAN and NAG strains of the shrews. The pointed part of the column represents the day the character first appeared; the full column marks the day the character was observed in all shrews. (N) indicates number of shrews examined. There were no appreciable differences between sexes for any features.

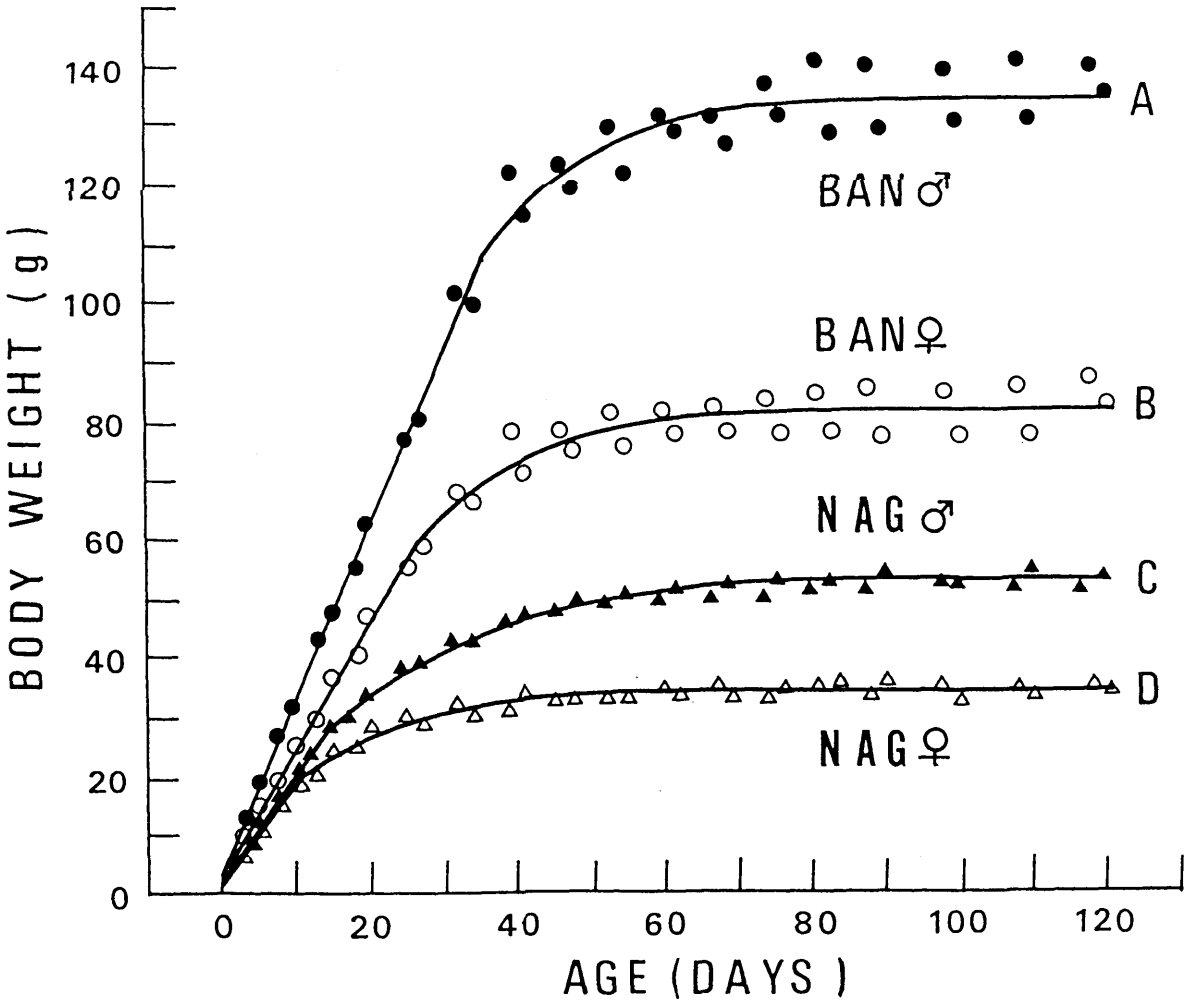

Fig. 5-2. Postnatal body weight gains of BAN and NAG strains of the shrews. Each dot represents the average body weight of respective groups (see Table 5-2 for details). Each of growth equations A, B, C, and D was composed of two parts: the linear and the decaying exponential equations. Those equations are as follows:

A) $W(t) = 3.0t + 2.7$, $(0 \leqq t \leqq 34, R^2=0.998)$,
$W(t) = 135.0(1 - e^{1.0325-0.0728t})$, $(34 \leqq t \leqq 120, R^2=0.876)$;

B) $W(t) = 2.1t + 3.5$, $(0 \leqq t \leqq 25, R^2=0.996)$,
$W(t) = 81.8(1 - e^{0.8800-0.0821t})$, $(25 \leqq t \leqq 120, R^2=0.937)$;

C) $W(t) = 1.7t + 2.2$, $(0 \leqq t \leqq 15, R^2=0.992)$,
$W(t) = 52.9(1 - e^{-0.0454-0.0466t})$, $(15 \leqq t \leqq 120, R^2=0.992)$;

D) $W(t) = 1.6t + 2.2$, $(0 \leqq t \leqq 10, R^2=0.998)$,
$W(t) = 33.5(1 - e^{-0.0682-0.0728t})$, $(10 \leqq t \leqq 120, R^2=0.980)$;

where $W(t)$ is body weight at age (t days); e is base of natural logarithms. Details of the generalized growth equation are described in the text.

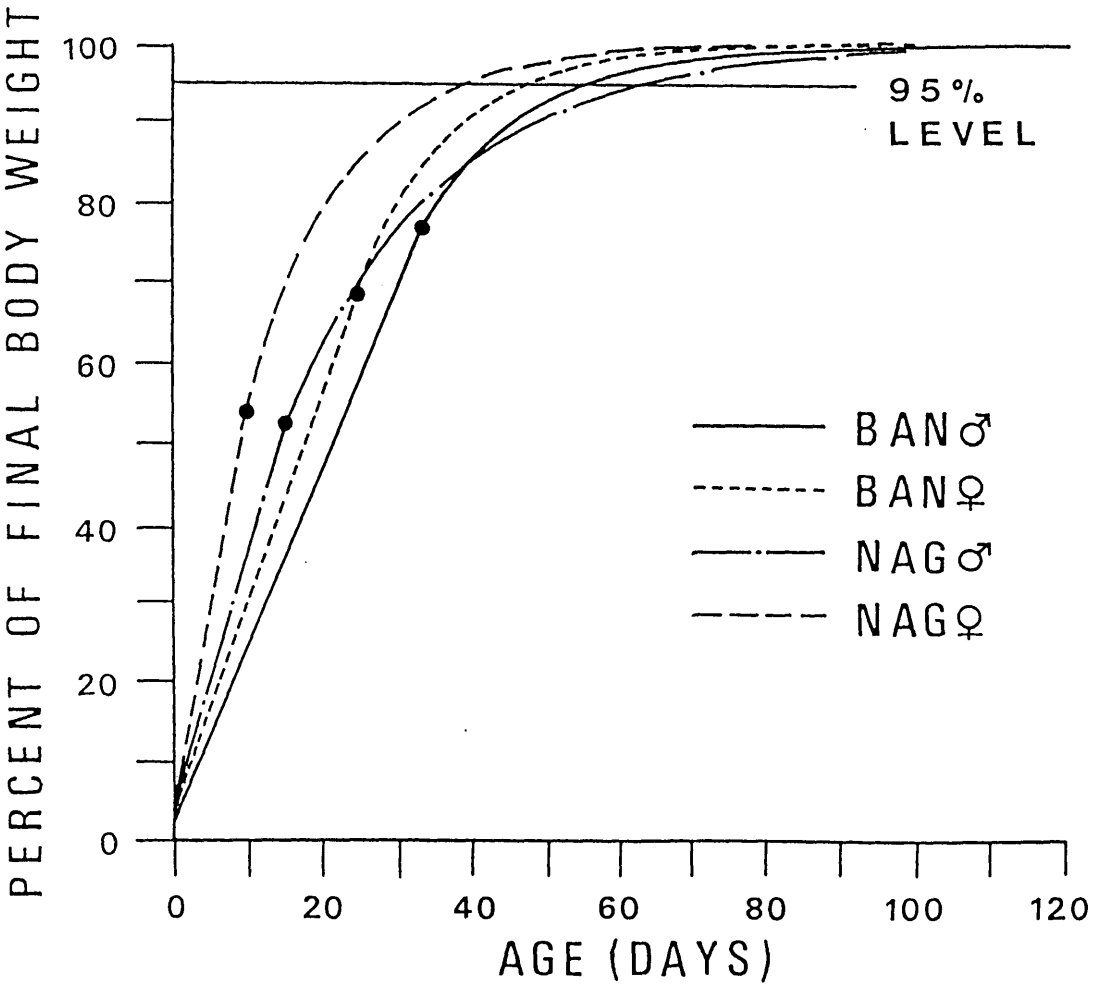

Fig. 5-3 Relative growth to final body weight (100%)obtained as an asymptotic weight from each growth equation given in Fig. 5-2. Solid circles indicate the end of the linear growth phase for the four groups (see Fig. 5-2 legend). The horizontal line represents 95% of final body weight; this point was defined as the end of the decaying growth phase or the growth phase.

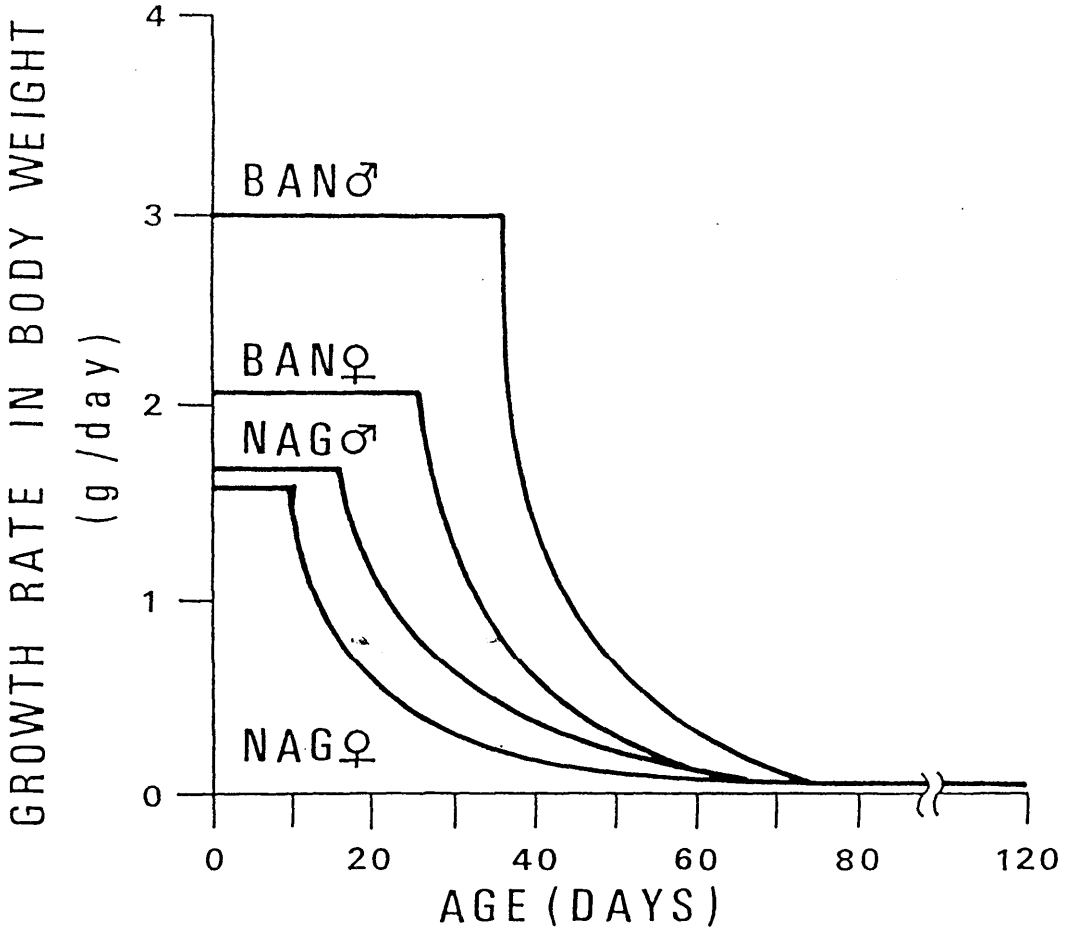

Fig. 5-4. Changes in postnatal growth rates in BAN and NAG strains of the shrews. These line-curves were obtained by differentiating the growth equation given in Fig. 5-2.

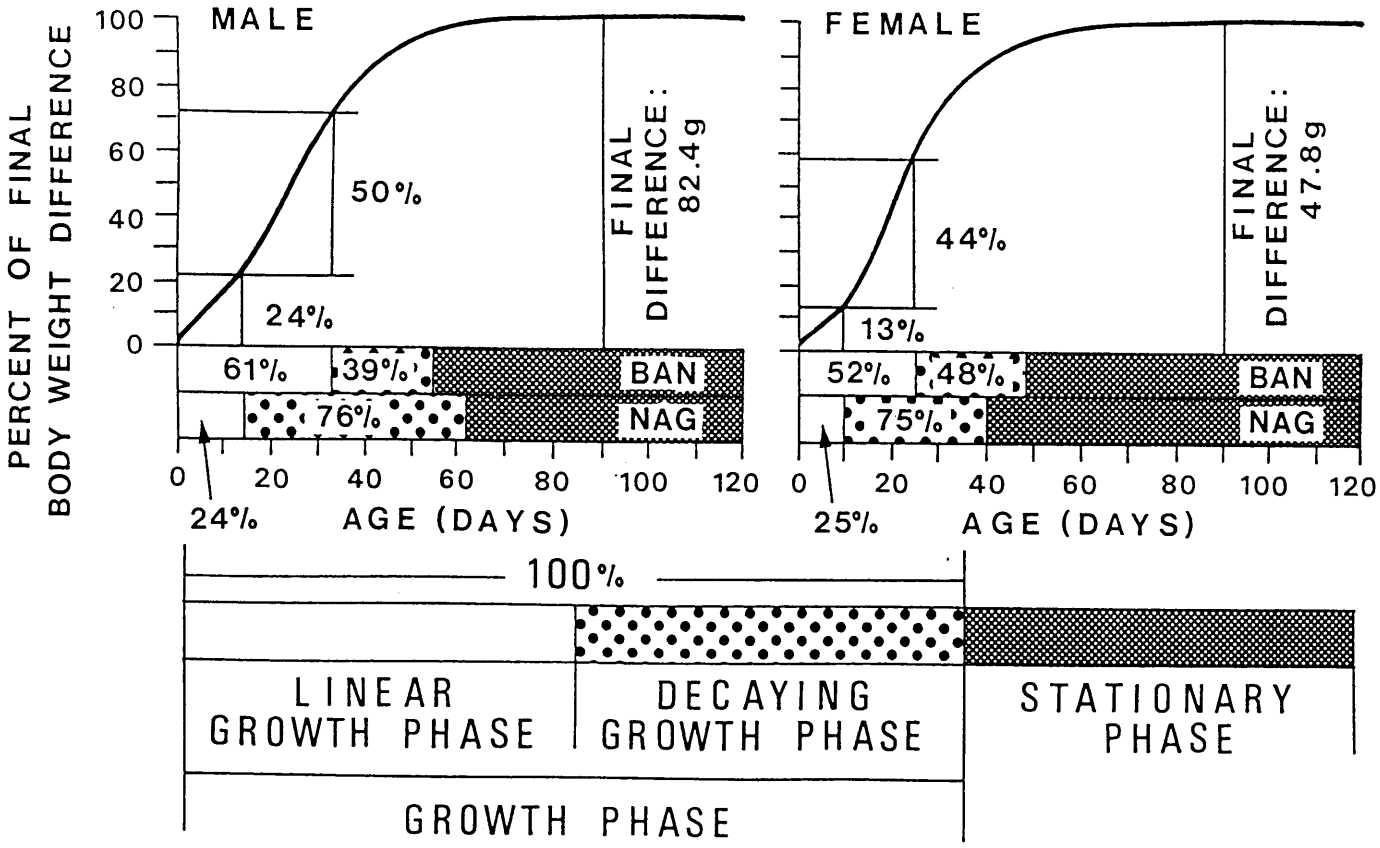

Fig. 5-5. Relationship of differences in body weight to differences in the growth process in BAN and NAG strains of the shrews. The body weight differences were obtained as relative values to differences in final body weight (100%), which were calculated from growth equations given in Fig. 5-2. Definitions of each growth phase and stationary phase were explained in the text.

Chapter VI.

Postnatal growth pattern of F_1 hybrids of a cross between BAN and NAG strains

INTRODUCTION

In Chapter V, it was shown that 120-day adult body weight in the BAN strain of the shrew is 135.3 g in males and 82.0 g in females, whereas for the NAG strain the respective values are 52.9 and 34.2 g, and this weight difference between the two strains reflects both an approximately 2.5 times longer duration of linear growth phase and an approximately 1.5 times higher growth rate during this phase in the BAN strain.

In the present chapter, I describe postnatal growth pattern of the F_1 hybrids, previously obtained by crossing large BAN males and small NAG females, using the growth characters mentioned in Chapter V (duration of linear growth phase, growth rate during this phase, etc.) as key characters. I also attempt to assess genetic differences in the growth patterns of these shrews.

MATERIALS AND METHODS

Detailed histories of BAN and NAG strains of the shrews have been provided in Chapter II and by Oda and Kondo (1976), respectively. The BAN shrews, used to obtain F_1 hybrids, consisted of 18 individuals taken

from first to fifth generation after capture; the NAG shrews consisted of 20 individuals from second to fifth generation after the strain was introduced into my laboratory from the Research Institute of Environmental Medicine, Nagoya University in 1983. Single-pair crosses between the two strains were conducted during a 2-day period. Eight male and 8 female F_1 hybrids were produced as a result of 6 out of a total of 11 mating trials between 6 BAN males and 8 NAG females. Details of the interbreeding experiments have been described elsewhere (Chapter IV).

The F_1 shrews and their parental strains were maintained in the same husbandry conditions described in Chapter V.

The day of birth was designated day zero after birth. Litter size was determined at 5 days of age, because it has been my experience that handling of pups during the first 5 days after birth often induces maternal cannibalism. After weaning (20 days of age), young shrews were placed in individual cages. The body weight of the F_1 hybrids was measured (to the nearest 0.01 g) at 5-day intervals from days 5 to 20, every 7 days to day 90, and at 10-day intervals to day 120.

The growth equation composed of linear and decaying exponential functions I employed in Chapter V was computed to fit the average weight data of littermates of the F_1 shrews of each sex, because the shrews were

not identified individually before weaning. Details of the generalized growth equation are given in Chapter V.

The growth equations for BAN and NAG strains were recalculated based on the growth data reported earlier (Chapter V) to allow parallel comparison of the growth characteristics of the F_1 shrews in the present study with those of the individual parental strains. The growth data for the strains were taken as representative of the parental strains I used in the present study, because most of the two parental strain shrews were derived from the same generations as in the previous study (Chapter V). In addition, there were no significant differences in litter size between the F_1 hybrids ($\bar{X}\pm SD$ 2.7±1.0, range 1 to 4 for 6 litters) and the individual parental strains (BAN: 3.0±1.2, 1 to 5, 23 litters; NAG: 2.7±1.3, 1 to 5, 30 litters). This suggests that the influence of litter size on postnatal growth is unimportant in terms of comparing them.

I have previously demonstrated that the combined growth equation provides the best description of the growth data in shrews of both BAN and NAG strains, because their body weights increase at relatively constant rates for several weeks after birth, followed by a period during which growth proceeds at progressively diminishing rates; the coefficient of determination (R^2) for the growth equations fitted to the strains ranges from 0.992 to 0.998 for the linear

function and from 0.877 to 0.991 for the decaying exponential function (Chapter V). In the present study, the R^2 value for the recalculated growth equation in the BAN strain was 0.995±0.005 ($\bar{X}$±SD) for the linear function and 0.732±0.195 for the decaying exponential function, whereas in the NAG strain the respective R^2 values were 0.994±0.004 and 0.830±0.124. My previous explanation also fits the growth data for the F_1 hybrids very well, because the R^2 value for the linear function was 0.997±0.03 and for the decaying exponential function 0.752±0.206.

The five characters derived from each growth equation analyzed for the F_1 hybrids and their parental strains consisted of asymptotic body weight (A), duration of the growth phase (DGP), duration of the linear growth phase ($DLGP$), growth rate during the linear growth phase (GR) equal to the slope of the linear function, and percentage of asymptotic body weight at the end of the linear growth phase (PA). The growth phase was defined as the period from birth to the day when the shrew reached 95% of its asymptotic body weight; the linear growth phase was defined as the period during which growth rates were constant for several weeks after birth or the period expressed by the linear function. Details of these definitions have been described in Chapter V. Phenotypic correlations among the five growth traits and litter size were

analyzed using the regression lines in each of the six sex-subclasses.

Statistical comparisons were made using t-test or Cochran-Cox test.

RESULTS

One NAG strain dam died during lactation (15 days after parturition), but her F_1 hybrid offspring successfully grew to adulthood. This maternal death of a small NAG parent may have been the result of exhaustion from having to provide large hybrid pups with large volumes of milk. Moreover, during the preweaning period the F_1 pups usually appeared rather slender. Consequently, these observations suggest that the results of the growth analysis of the F_1 hybrids described below may have been somewhat influenced by nursing conditions.

Body weight data of the F_1 hybrids from 5 to 120 days of age are presented in Table 6-1. From 5 to 15 days of age, the mean body weight of F_1 females tended to be consistently higher than that of F_1 males, but the differences in weight at the respective ages were not statistically significant. This is evidence in support of the undernourishment of the F_1 hybrids, especially the males, mentioned above, because males generally tend to be heavier than females from birth onwards (Chapter V; Dryden, 1968; Oda and Kondo, 1976).

At weaning (20 days of age), the F_1 males averaged 39.9 g (range 31.0 to 52.0 g) and the F_1 females 38.9 g (33.2 to 46.1 g). At 27 days of age, the body weight of the sexes differed significantly ($P<0.05$), and this weight difference grew increasingly with age.

Figure 6-1 shows the body-weight growth curves of F_1 hybrids, in comparison with their parental strains, BAN and NAG. The growth trajectories of both sexes of F_1 hybrids were situated roughly midway between those of the parental strains. At 120 days of age, F_1 males weighed 86.0 g (range 70.6 to 103.6 g) and females 51.7 g (42.9 to 56.1 g). In contrast, BAN males weighed 135.3 g (range 112.7 to 173.2 g) and females 82.0 g (67.6 to 115.7 g); NAG shrews weighed 52.9 g (47.0 to 58.5 g) and 34.2 g (30.2 to 33.8 g), respectively.

Table 6-2 shows characteristics of the five traits derived from the growth curve equation for the F_1 hybrids and their parental strains, BAN and NAG. Estimated asymptotic body weight (*A*) nearly matched with the above-mentioned 120-day body weight in each subclass. The duration of the growth phase (*DGP*) did not differ significantly among F_1 hybrids and the two parental strains in the individual sexes. However, the BAN shrews of both sexes exhibited both a significantly longer duration of linear growth phase (*DLGP*) and a significantly higher growth rate during the linear

growth phase (*GR*) than the NAG shrews. The estimates for these two traits in the F_1 hybrids corresponded approximately to mid-parent values and were significantly greater than those of NAG shrews. The percentage of asymptotic body weight at the end of the linear growth phase (*PA*) exhibited the very same tendency as *DLGP* and *GR* apart from the results of the statistical comparisons. There were no significant differences in the *PA* of the sexes in each subclass in comparison with the other traits. It is clear that *DLGP* and *GR* reflect genetic differences in the growth patterns of the subclasses.

Phenotypic correlations among the five growth characters and litter size calculated within subclasses are presented in Table 6-3. In the four or five of the six subclasses, there were significant positive correlations both between *A* and *GR* and between *DLGP* and *PA*. However, *A* was not significantly correlated with *DGP*, *DLGP* or *PA* in any of the subclasses. This suggests that differences in asymptotic body weight (*A*) within each subclass may be caused by differences in growth rate during the linear growth phase (*GR*). Hardly any growth characters were significantly correlated with litter size (*LS*) with the same sign in the subclasses.

DISCUSSION

This is the first study to demonstrate the genetic difference in postnatal growth between large BAN shrews and small NAG shrews. The present results of growth analysis of the F_1 hybrids in comparison with their parental strains revealed that changes in the growth pattern can be explained on the basis of only three growth characters, duration of growth phase (*DGP*), duration of linear growth phase (*DLGP*), and growth rate during the linear growth phase (*GR*).

The growth traits reported for different body-sized musk shrews have been classified on the basis of my categories to compare with the present results and are summarized in Table 6-4. For each sex, the duration of the growth phase (*DGP*) in BAN and NAG strains and their F_1 hybrids is nearly identical with that reported for small-bodied shrews from Japan and smaller-bodied shrews from Guam, meaning that neither body size nor geographical origin appear to affect growth period. Hence, this trait is obviously sex-dependent in this species: this period lasts about 60 days in males and about 40 days in females.

BAN shrews of both sexes, with the highest adult body weight in this species, have the longest duration of the linear growth phase (*DLGP*). In NAG shrews and cross-bred Japanese shrews, both having small body size, on the other hand, this period lasts only approximately half as long as in the BAN strain. The

F_1 hybrids, possessing a body weight intermediate between BAN and Japanese shrews, fall between them in terms of this period. The growth rate during the linear growth phase (*GR*) exhibits precisely the same tendency as the duration of this phase (*DLGP*). It is clear that the two characters contribute greatly to genetic differences in the growth patterns of the different size shrews and the sexes.

In the laboratory mouse, the parameter estimates of such sigmoid growth curves as the logistic, Gompertz and Bertalanffy equations play a very important role in understanding the genetics of postnatal growth, details of which are reviewed by Eisen (1974 and 1976). The growth curve parameters such as age at point of inflection and asymptotic body weight are of biological significance and can quantitatively describe differences in the growth patterns of inbred lines, lines selected for postweaning weight gain, and the sexes (Eisen *et al.*, 1969; Laird and Howard, 1967; Timon and Eisen, 1969). Kachman *et al.* (1988) suggest that growth curve parameters may be useful in selecting for rapid early growth. In the musk shrew, I found that two characters derived from the growth curve (*DLGP* and *GR*) are chiefly responsible for the strain and sex differences in postnatal growth pattern. These two growth characters therefore appear to be genetic components, like the growth curve parameters of

mice, important to understanding the inheritance of growth patterns in the shrews. Estimates of direct genetic effects on the growth characters will provide more precise information with regard to this matter.

SUMMARY

In my previous comparison of the postnatal growth of BAN (large size) and NAG (small size) strains of the shrews, I found that the difference in the 120-day adult body weight of the two strains (BAN: males 135.3 g and females 82.0 g; NAG: 52.9 and 34.2 g, respectively) reflect both an approximately 2.5 times longer duration of linear growth phase and an approximately 1.5 times higher growth rate during this phase in the BAN strain. I examined the postnatal growth (from 5 to 120 days after birth) of the F_1 hybrids of a cross between the two strains and compared my findings with the above-mentioned growth data for the parental strains. A growth equation composed of linear and decaying exponential functions was fitted to the growth data for the shrews. The growth trajectories of both sexes of the F_1 hybrids were situated roughly midway between those of the two parental strains throughout the entire growth process. The adult body weight of the F_1 shrews at 120 days of age averaged 86.0 g in males and 51.7 g in females. The growth phase lasted

approximately 60 days in males and approximately 40 days in females of both the F_1 shrews and the parental strains. The duration of the linear growth phase of the F_1 hybrids (29.8 days in males and 19.0 days in females), however, was approximately intermediate between the parental strains (BAN: males 33.4 days and females 26.1 days; NAG: 18.8 and 13.9 days, respectively). Likewise, growth rates during the linear growth phase of the F_1 shrews (males 2.1 g/day and females 1.8 g/day) were roughly intermediate between those of the parental strains (BAN: males 3.2 g/day and females 2.2 g/day; NAG: 1.6 and 1.5 g/day, respectively). The positive phenotypic correlation between growth rate and asymptotic body weight in the growth equation was significantly high in four of six sex-subclasses. It is clear that characteristics of both the duration of linear growth phase and the growth rate during this phase reflect genetic differences in the growth patterns of the shrews.

Table 6-1. Postnatal changes in body weight (g) of F_1 *hybrids between BAN and NAG strains of the shrews.*

Age	Male		Female	
(days)	*n*	$\bar{X}\pm SD$	*n*	$\bar{X}\pm SD$
5	6	10.9± 3.4	5	12.6±2.4
10	6	20.9± 5.0	5	22.5±4.0
15	8	28.1± 5.3	7	30.4±4.1
20	8	39.9± 7.4	7	38.9±4.8
27	8	54.2± 9.2	7	44.3±5.5
34	8	65.1±12.0	7	47.3±7.1
41	6	76.1±12.8	6	50.1±9.4
48	8	76.9±12.5	7	51.4±9.2
55	8	80.4±13.0	7	52.0±8.9
62	8	81.8±13.1	7	53.5±7.9
69	8	82.8±12.3	7	52.5±6.3
76	8	82.2±12.0	7	51.4±5.3
83	7	82.1±12.2	7	51.8±5.1
90	8	83.7±15.0	7	51.6±4.6
100	8	83.2±14.5	7	51.6±4.9
110	8	83.7±14.0	7	52.5±4.3
120	8	86.0±13.9	7	51.7±4.9

Note: F_1 hybrids were obtained only by crossing of BAN males to NAG females (see Chapter IV for details).
n: Number of animals weighed.

Table 6-2. Characteristics of the five traits derived from the growth equations for F_1 shrew hybrids and their parental strains, BAN and NAG.

Subclass	*n*	Trait# *A* (g)	*DGP* (days)	*DLGP* (days)	*GR* (g/day)	*PA* (%)
BAN male	14	136.8±23.2^{a*}	58.4±16.8^{a*}	33.4±7.0^{a*}	3.2±0.6^{a*}	77.2± 9.9^{a}
F_1 male	5	86.9±13.9^{b*}	57.3±15.6^{a}	29.8±3.8^{a*}	2.1±0.4^{b}	71.0±10.0^{ab}
(Mid-parent value)		(94.7)	(59.4)	(26.1)	(2.4)	(70.4)
NAG male	15	52.6± 3.6^{c*}	60.3±21.6^{a*}	18.8±5.5^{b*}	1.6±0.3^{c}	63.5±14.9^{b}
BAN female	13	81.3±10.2^{a}	46.9±10.0^{a}	26.1±4.8^{a}	2.2±0.4^{a}	74.9± 8.8^{a}
F_1 female	5	52.9± 6.1^{b}	48.2±13.2^{a}	19.0±2.2^{b}	1.8±0.3^{a}	70.6± 8.6^{a}
(Mid-parent value)		(57.9)	(45.9)	(20.0)	(1.9)	(71.2)
NAG female	12	34.4± 2.4^{c}	44.9± 7.1^{a}	13.9±2.6^{c}	1.5±0.2^{b}	67.5±11.5^{a}

Note: All values indicate the mean±*SD*.
#: *A*-Asymptotic body weight; *DGP*-Duration of growth phase, from birth to the day when the shrew reached a body weight plateau; *DLGP*-Duration of linear growth phase, expressed by the linear function (broken line) in Fig. 6-1; *GR*-Growth rate during the linear growth phase, equal to the slope of the linear function (Fig. 6-1); *PA*-Percentage of asymptotic body weight at the end of the linear growth phase.
n: Number of litters fitted into the growth equations.
a-c: Means with the same superscript letters within each column in each sex are not significantly different at $P<0.05$.
*: Significantly different from females at $P<0.05$.

Table 6-3. Phenotypic correlations among the five growth characters and litter size in F_1 shrew hybrids and their parental strains, BAN and NAG.

Traits	Male			Female		
correlated#	BAN	F_1	NAG	BAN	F_1	NAG
A - DGP	0.32	0.20	0.27	0.52	0.34	0.06
A - DLGP	0.20	-0.43*	-0.43*	-0.07	-0.56	-0.12
A - GR	0.78***	0.95*	0.56*	0.57*	0.75	0.29
A - PA	0.18	-0.23	-0.33*	-0.38	-0.75	-0.13
A - LS	-0.13	-0.11	-0.56**	0.11	-0.38	-0.35*
DGP - DLGP	0.20	-0.70	-0.71**	-0.06	0.12	-0.63*
DGP - GR	0.03	0.11	-0.07***	0.02	-0.29	-0.15**
DGP - PA	-0.19*	-0.66*	-0.87***	-0.07	-0.41	-0.79**
DGP - LS	0.57*	-0.91*	-0.04	0.54**	-0.09	0.65*
DLGP - GR	-0.38***	-0.29**	-0.44***	-0.74*	-0.68	-0.20**
DLGP - PA	0.83***	0.97**	0.89***	0.62*	0.45*	0.81**
DLGP - LS	-0.06	0.65	0.34	0.30	0.92*	-0.38
GR - PA	-0.18	-0.13	-0.03**	-0.43	-0.27	0.33
GR - LS	-0.08	0.09	-0.68**	-0.44	-0.35	-0.55*
PA - LS	-0.31	0.43	0.07	-0.28	0.42	-0.65*

#: *LS*-Litter size; other abbreviations are explained in the footnote to Table 6-2.
*: $P<0.05$.
**: $P<0.01$.
***: $P<0.001$.

Table 6-4. Characteristics of the three growth traits (*DGP*, *DLGP*, and *GR*) in different-sized shrews derived from various geographical areas.

Origin	Adult body weight (g)	Trait[#] *DGP* (days)	*DLGP* (days)	*GR* (g/day)	Reference
Male					
Bangladesh (BAN strain)	135.3	58.4[a]	33.4[a]	3.2[a]	Chapter V
F_1 (NAG X BAN)	86.0	57.3	29.8	2.1	Present data
Nagasaki, Japan (NAG_b strain)	52.9	60.3[a]	18.8[a]	1.6[a]	Chapter V
Japan[b]	65.3	63	15[a]	1.8[a]	Furumura *et al.* (1984)
Okinawa, Is., Japan	50.5	56			Shigehara (1980)
Guam	45-50	60			Dryden and Ross (1971)
Female					
Bangladesh (BAN strain)	82.0	46.9[a]	26.1[a]	2.2[a]	Chapter V
F_1 (NAG X BAN)	51.7	48.2	19.0	1.8	Present data
Nagasaki, Japan (NAG_b strain)	34.2	44.9[a]	13.9[a]	1.5[a]	Chapter V
Japan[b]	39.2	42			Furumura *et al.* (1984)
Okinawa, Is., Japan	33.8	42			Shigehara (1980)
Guam	25-30	40			Dryden and Ross (1971)

#: Abbreviation of the traits are explained in the footnote to Table 6-2.
a: Values recalculated by the present method.
b: Cross-bred shrews originating from wild animals in Nagasaki, Okinawa, Is., and Tokunoshima Is., Japan.

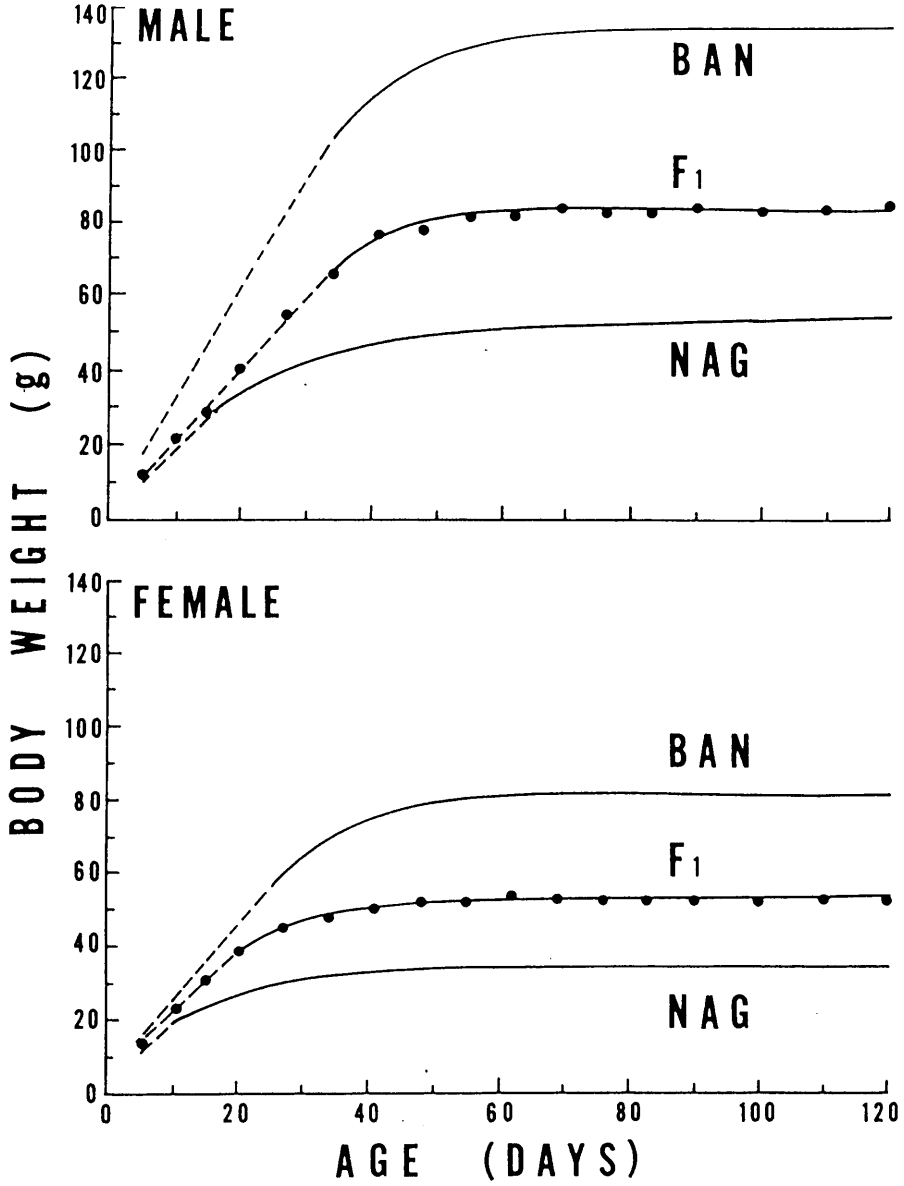

Fig. 6-1. Postnatal body-weight growth curves of F_1 musk shrew hybrids and their parental strains, BAN and NAG. The dots represent average body weights (see Table 6-1 for details). Each growth equation is composed of two parts, the linear (broken line) and the decaying exponential (solid line) functions. The equations for the two parental strains are quoted from Chapter III. The equations of the F_1 hybrids are as follows:

Males: $W(t) = 1.3 + 1.9t$, $(0 \leqq t \leqq 34)$,

$W(t) = 83.4(1 - e^{2.1030 - 0.1084t})$,

$(34 \leqq t \leqq 120)$;

Females: $W(t) = 4.4 + 1.7t$, $(0 \leqq t \leqq 20)$,

$W(t) = 52.2(1 - e^{0.1802 - 0.0784t})$,

$(20 \leqq t \leqq 120)$;

where $W(t)$ is body weight at age t (days); e is base of natural logarithms. Details of the generalized growth equation are described in Chapter V.

Chapter VII

The inheritance of body weight in BAN and NAG strains: an estimate of the minimum number of genes for the weight difference

INTRODUCTION

In Chapter IV, mating tests between BAN and NAG strains showed that F_1, F_2, and backcross hybrids can be obtained by crossing the large BAN males with the small NAG females, but no hybrid generations of the small NAG males and the large BAN females are produced. This difference in mating success is caused by the difference in body weight between the sexes paired, which is believed to be a partial reproductive isolation mechanism existing the two strains derived from different geographical races (Chapter IV). In a previous comparison of postnatal growth of the two strains under the same laboratory conditions, I demonstrated that the difference in their adult body weight reflects both an approximately 2.5 times longer duration of linear growth phase and an approximately 1.5 times higher growth rate during this phase in the BAN strain (Chapter V). In the subsequent study on postnatal growth of the F_1 hybrids from a cross between the large BAN males and the small NAG females, I revealed that both the duration of linear growth phase and the growth rate during this phase are controlled by genetic

factors (Chapter VI).

In the present chapter, I describe the distribution of adult body weight for the F_1, F_2, and backcross shrew hybrids produced from a cross between the large BAN males and the small NAG females to elucidate the inheritance of the weight. In addition, I attempt to estimate the minimum (or effective) number of genes contributing to the body weight difference of the two strains from the means and variances of the two parental strains and their F_1, F_2, and backcross hybrids using statistical techniques of quantitative genetics.

MATERIALS AND METHODS

Historical developments of BAN and NAG strains of the shrews have been reported in Chapter II and by Oda and Kondo (1976), respectively. In my previous mating tests of BAN males and NAG females, a total of 141 shrew hybrids consisting of F_1, F_2, and first-backcross generations were produced (Chapter IV). The mating combinations, designations for progeny groups, and average litter size at 5 days after birth are given in Table 7-1. Husbandry conditions for the shrews have been reported elsewhere (Chapter II).

The body weight of the hybrids was measured (to the nearest 0.01 g) at 120 days of age, the day when the shrew attained a full body-weight plateau (Chapter

V). Weight data for two parental strains, BAN and NAG, were quoted from my previous study (Chapter V) to allow parallel comparison with those of the hybrids. These data were representative of the present parental strains, because most of the parental shrews were derived from the same generations as in Chapter V.

RESULTS AND ANALYSIS

Distribution of Body Weight

Adult body weight at 120 days of age in respective sexes of each hybrid generation is shown in Table 7-2. The average body weight of males was heavier than that of females in each generation. Such sex differences are generally observed in this species (Chapter IV). In order to minimize the complication of the sex differences and the effect of small sample sizes of progeny groups in the later calculations of quantitative genetics, females weights were adjusted to male-equivalents using the following correction equation reported in Fig. 4-2, Chapter IV:

$$Y = -4.67 + 1.70X$$

where X is an original female weight; Y is the adjusted weight.

In addition to the sex differences, each hybrid group was produced by mothers which were greatly different in body weight (Tables 7-1 and 7-2). Since F_1 hybrid mothers were reported to have higher repro-

ductive performance than the parental strains, BAN and NAG (Chapter IV), their maternal effects to the young appeared to act greater than those of BAN and NAG mothers. Similarly the large BAN mother was likely to have more maternal effects than the small NAG mother.

Moreover, litter size was not standardized in the present study. Mean litter sizes in F_2, B_{1-1}, and B_{2-1} shrews, which were all born to F_1 hybrids as the maternal parents, were larger than those of the other progeny groups and the ranges leaned toward larger litter sizes (Table 7-1). In each progeny group, the analysis of variance of 120-day body weight indicated that the variation due to litter size, in which certain maternal effects were considered to be apparently involved, was not significant in the F_1 (73.8% of a total variance), F_2 (59.2%), and B_{2-2} (55.6%) hybrid groups, whereas that was significant ($P<0.05$) in the P_1 (80.0%), P_2 (88.2%), B_{1-1} (81.9%), B_{1-2} (81.7%), and B_{2-1} (84.2%) shrew groups. Hence the 120-day weight within and between the shrew groups seems to be unequally affected by litter size, as well as the maternal effects mentioned above.

Figure 7-1 illustrates the distribution of 120-day adult body weight on the simple logarithmic scale in each generation pooled for individual sexes. The reason for using the logarithmic scale will be discussed later (under *Choice of a Suitable Scale*). The

mean body weights of F_1 and F_2 hybrids were approximately intermediate between those of two parental shrews, P_1 and P_2. The means in reciprocal backcrosses, B_1 and B_2, were approximately halfway between those of the respective parents. The segregating generations, F_2 and reciprocal backcross, gave unimodal distributions for body weight, but in the F_2 shrews a small, extra peak was observed around the body weight of 125 g. However there was no evidence that a single major gene controls the weight variation of the shrews.

Choice of a Suitable Scale

It is very much important for the analysis of quantitative variability to choose an adequate scale, on which the mean and variance are independent from each other and non-additive interactions caused by genetic and/or environmental factors are removed or reduced (Falconer, 1981; Wright, 1968). On a suitable scale, the means of two parents, P_1 and P_2, and their F_1, F_2, and reciprocal backcross (B_1 and B_2) generations are expected to be as follows:

$$\bar{B}_1 = 1/2(\bar{F}_1 + \bar{P}_1)$$

$$\bar{B}_2 = 1/2(\bar{F}_1 + \bar{P}_2)$$

$$\bar{F}_2 = 1/2(\bar{F}_1 + \bar{P}_M)$$

where the subscript M denotes mid-parent.

The suitability of the chosen scale can be tested by observed differences between both sides of the above equations, expressed as *A*, *B*, and *C* (see below), the

tests have been termed "scaling tests" by Mather and Jinks (1977):

$A = 2\bar{B}_1 - \bar{P}_1 - \bar{F}_1 \qquad V_A = 4V_{\overline{B1}} + V_{\overline{P1}} + V_{\overline{F1}}$

$B = 2\bar{B}_2 - \bar{P}_2 - \bar{F}_1 \qquad V_B = 4V_{\overline{B2}} + V_{\overline{P2}} + V_{\overline{F1}}$

$C = 4\bar{F}_2 - 2\bar{F}_1 - \bar{P}_1 - \bar{P}_2 \qquad V_C = 16V_{\overline{F2}} + 4V_{\overline{F1}} + V_{\overline{P1}} + V_{\overline{P2}}$

where V_A, V_B, and V_C are variances for *A*, *B*, and *C*, respectively, calculated from variances of the means for respective groups. The standard error is obtained from the square root of V_A, V_B, or V_C. The expected values of *A*, *B*, and *C* are all zero. Tests of significance are performed using a table of normal deviates in the customary way.

In the present analysis, I adopted three scales, the arithmetic, the simple logarithm to the base 10, and the modified logarithm defined by Wright (1968). Body weight data on the three scales in each generation are shown in Table 7-3. Among P_1, P_2, and F_1 generations, the variances of body weight differed significantly in all three possible combinations on the arithmetic scale and in two combinations on the simple logarithmic scale. This means that the mean and variance were not independent from each other. However, such significant differences were not observed for the data transformed to the modified logarithm (Table 7-3).

Table 7-4 indicates the results of scaling tests for the weight data on the three scales. On the

arithmetic scale, all three tests (*A*, *B*, and *C*) were not significantly different from zero. On the other hand, there were significant differences from zero in one or two scaling tests on the two logarithmic scales.

Combining the two results of the statistical analyses described above suggests that the simple logarithmic scale is more adequate than the arithmetic and modified logarithmic scales because it satisfies the early-mentioned assumptions approximately. Therefore only the means and variances on the simple logarithmic scale were considered in estimating the minimum number of gene.

Minimum Number of Genes

For estimating the minimum number of genes (or freely segregating loci), the following assumptions should be held: 1) two parental populations are homozygous at the relevant loci; 2) all the loci are unlinked and act additively with equal allelic effects. Details of these assumptions have been mentioned by Lande (1981) and Wright (1968).

For the first assumption, since two parental strains, BAN and NAG, employed in the present study are derived from wild shrew populations which have probably diverged by natural selection for adult body weight (Chapter II; Oda and Kondo, 1976), it can be considered that the BAN shrew contains all the effective alleles increasing body weight and the NAG shrew contains all

the decreasing alleles. The additive gene action, one of the second assumptions, has already been acquired by making use of the simple logarithmic scale, as discussed in the above section.

The Wright-Castle formula (Castle, 1921; Wright, 1968) has widely been used for estimating the minimum number of genes affecting a quantitative character. In the present study, I used the modified procedure of the Wright-Castle method, proposed by Cockerham (1986). This is generalized to genetically heterogeneous parental populations as well as inbred strains. The estimate of the gene number (N) is expressed by

$$N = \frac{(\bar{P}_1 - \bar{P}_2)^2 - (V_{\bar{P}1} + V_{\bar{P}2})}{8V_s}$$

where $\bar{P}_1$ and $\bar{P}_2$ are means of two parents; $V_{\bar{P}1}$ and $V_{\bar{P}2}$ are variances of the parental means; V_s is the additive genetic variance estimated from variances of two parents and the F_1, F_2, and backcross progeny using the least squares method. The calculation method of the standard error of N is also provided in the Cockerham's procedure.

From the analysis of the weight data on the simple logarithmic scale, it was estimated that a minimum of 8.0±2.9 ($\pm SE$) genes contribute to the difference in adult body weight between BAN and NAG strains.

DISCUSSION

The distribution of 120-day adult body weight in hybrids from a cross between the large BAN and small NAG shrews gave the evidence that the weight variation is under the polygenic control. The biometrical analysis of the body weight on the simple logarithmic scale revealed that the weight difference of the two strains is controlled by about 8 freely segregating genes. This is the first estimate of this kind to have been calculated in this species.

The gene number calculated is considered to be a rough estimate in two respects. First, environmental variances of 120-day body weight caused by differences in both maternal effects and litter size were not identical among two parental strains, BAN and NAG, and their hybrid generations (Table 7-1). Second, sample sizes of the hybrid generations (Table 7-3) were smaller than those providing the accurate estimation of the gene number, which were in need of at least 20 or 30 individuals in the F_1 population and around 100 or more in the F_2 and backcross populations (Lande, 1981).

It may not be worthwhile making every efforts to estimate more accurate number of genes in my further study, because it is well known that when the assumptions of equal allelic effects or unlinked loci are violated, the estimation procedure leads to the true gene number being substantially underestimated

(Lande, 1981; Falconer, 1981; Zeng *et al*., 1990). Zeng *et al*. (1990) have pointed out that even in the best of circumstances, information from the gene number estimated is very limited by the assumptions made during analysis.

Despite the above-mentioned limitations, the gene number estimated in the present study may play a very much important role in understanding the genetic basis of body weight in the shrews, because the estimate can be interpreted as average properties of a group of "quantitative trait loci" (QTLs) actually influencing the body weight difference of the BAN and NAG strains, as Paterson *et al*. (1991) have pointed out. In Chapter VI, I found that two postnatal growth characters, the duration of linear growth phase and the growth rate during this phase, contribute greatly to the genetic difference in growth pattern between the BAN and NAG strains. If each of the two growth characters is under separate genetic controls, the gene number affecting the respective characters would be substantially fewer than 8 genes roughly estimated for the body weight. This suggests that using the two growth characters as sub-components of body weight may make the genetic analysis of the body weight easier.

Recently, linkage analysis of molecular genetic markers and QTLs affecting a quantitative trait have made it possible to define the location and specific

phenotypic effects of the individual QTLs (Cowan *et al.*, 1990; Lander and Botstein, 1989; Keim *et al.*, 1990; Paterson *et al.*, 1988, 1990 and 1991; Tanksley and Hewitt, 1988). By means of this new technique, it may be possible to map individual polygenes controlling the two growth characters as well as the adult body weight in the large BAN and small NAG shrews at the molecular level. This will provide the information very much important to understand evolutionary changes and growth regulation systems in the adult body weight of this species, which is of the greatest interest to me.

SUMMARY

I have previously acquired the hybrid progeny of a cross between BAN (large size) and NAG (small size) strains of the shrews. Using the hybrid progeny obtained, I examined the inheritance of 120-day adult body weight in the two strains. Mean body weights of F_1 and F_2 generations were approximately intermediate between those of the two parental strains. The means of reciprocal backcross generations were approximately halfway between those of the respective parents. The distribution of the body weight was unimodal in the segregating generations, F_2 and reciprocal backcrosses, but a small, extra peak was observed in the F_2 shrews.

These provided the evidence that the variation of the body weight was under the polygenic control. The biometrical analysis of the weight data on the simple logarithmic scale indicated that a minimum of 8 freely segregating genes contributed to the difference in adult body weight between the extreme parental strains. Although the gene number calculated was considered to be a rough estimate because of 1) unequal contribution of environmental variance caused by both maternal effects and litter sizes in the two parental and the hybrid shrews; and 2) small sample sizes of the hybrid groups, the estimate provided fundamental information about mapping individual polygenes controlling the difference in the adult body weight of the two shrew strains.

Table 7-1. Mating combinations, progeny groups, and litter sizes at 5 days after birth in a cross between BAN and NAG strains of the shrews.

Mating combination	Abbreviated designation of progeny groups	Litter size		
Female X Male		*n*	$\bar{X}\pm SD$	Range
NAG X NAG*	P_1	30	2.7 ± 1.3^{a}	1-5
BAN X BAN*	P_2	23	3.0 ± 1.2^{ab}	1-5
NAG X BAN	F_1	6	2.7 ± 1.0^{ab}	1-4
F_1 X F_1	F_2	13	4.3 ± 1.0^{c}	3-6
F_1 X NAG	B_{1-1}	5	3.6 ± 1.1^{abc}	2-4
NAG X F_1	B_{1-2}	8	2.7 ± 0.5^{a}	2-3
F_1 X BAN	B_{2-1}	6	3.7 ± 0.5^{bc}	3-4
BAN X F_1	B_{2-2}	7	2.3 ± 1.1^{a}	1-4

Note: F_1 hybrids were obtained only by crossing NAG females with BAN males (see Chapter IV for details).
n: Number of cases observed.
*: Values reported in Chapter V.
a-c: Means with the same letters are not significantly different at $P<0.05$ (*t*-test or Cochran-Cox test).

Table 7-2. The 120-day adult body weight (g) of each generation in an interstrain cross of the shrews.

Generation	Male			Female		
	n	$\bar{X}\pm SD$	Range	*n*	$\bar{X}\pm SD$	Range
P_1	27	$52.9\pm3.6^{e*}$	47.0-58.5	16	$34.2\pm2.7^{g*}$	30.2-38.8
P_2	20	$135.3\pm18.5^{a*}$	112.7-173.2	28	$82.0\pm12.1^{e*}$	67.6-115.7
F_1	8	86.0 ± 13.9^{b}	70.6-103.6	7	51.7 ± 4.9^{ad}	42.9-56.1
F_2	21	101.7 ± 13.7^{c}	78.4-134.1	33	54.6 ± 6.7^{ac}	45.0-71.5
B_{1-1}	7	84.0 ± 10.7^{b}	70.7-95.5	10	48.2 ± 6.7^{d}	39.8-64.2
B_{1-2}	10	62.1 ± 6.4^{d}	55.7-76.0	10	40.7 ± 3.5^{f}	35.6-45.0
B_{2-1}	11	121.7 ± 22.2^{a}	81.6-156.6	11	62.9 ± 11.5^{bc}	37.1-77.8
B_{2-2}	7	13.68 ± 13.9^{a}	121.3-162.3	6	65.1 ± 4.8^{b}	57.7-71.6

*: Weight data reported in Chapter V.
n: Number of shrews examined.
a-g: Means with the same letters within each column for individual sexes are not significantly different at $P<0.05$ (*t*-test or Cochran-Cox test).

Table 7-3. The 120-day body weight ($\bar{X}\pm SD$) of overall groups on three scales in an interstrain cross of the shrews.

Generation	n	Scale#		
		x	log x	log(x-29.4)
P_1	43	53.1± 3.9[a]	1.7241±0.0371[a]	1.3699±0.0714[a]
P_2	48	134.9±19.3[b]	2.1260±0.0585[b]	2.0169±0.0739[a]
F_1	15	84.7±11.0[c]	1.9241±0.0557[b]	1.7341±0.0859[a]
F_2	54	93.4±13.2	1.9660±0.0619	1.7970±0.0903
B_1	37	71.0±12.0	1.8453±0.0699	1.6025±0.1187
B_2	35	115.9±21.6	2.0560±0.0864	1.9215±0.1236

Note: B_1 is composed of B_{1-1} and B_{1-2}; B_2 consists of B_{2-1} and B_{2-2}.

#: x: Arithmetic, log x: Simple logarithm to the base 10, log(x-29.4): Modified logarithm from Wright (1968).

n: Number of shrews composed of males and females (see Table 7-2).

a-c: Variances (SD^2) with the same letter among the P_1, P_2, and F_1 generations are not significantly different at $P<0.05$ (F-test).

Table 7-4. Scaling tests (*A*, *B*, and *C*) on the body weight data on the three scales shown in Table 7-3.

Scaling test	$\bar{X}\pm SE$		
	x	log x	log(x-29.4)
A	4.2±4.9	0.0424±0.0276	0.1010±0.0462*
B	12.2±8.3	0.0619±0.0336	0.0920±0.0485
C	16.3±9.8	0.1657±0.0452*	0.3330±0.0680*

*: Significantly different from the expected value of zero at $P<0.05$.

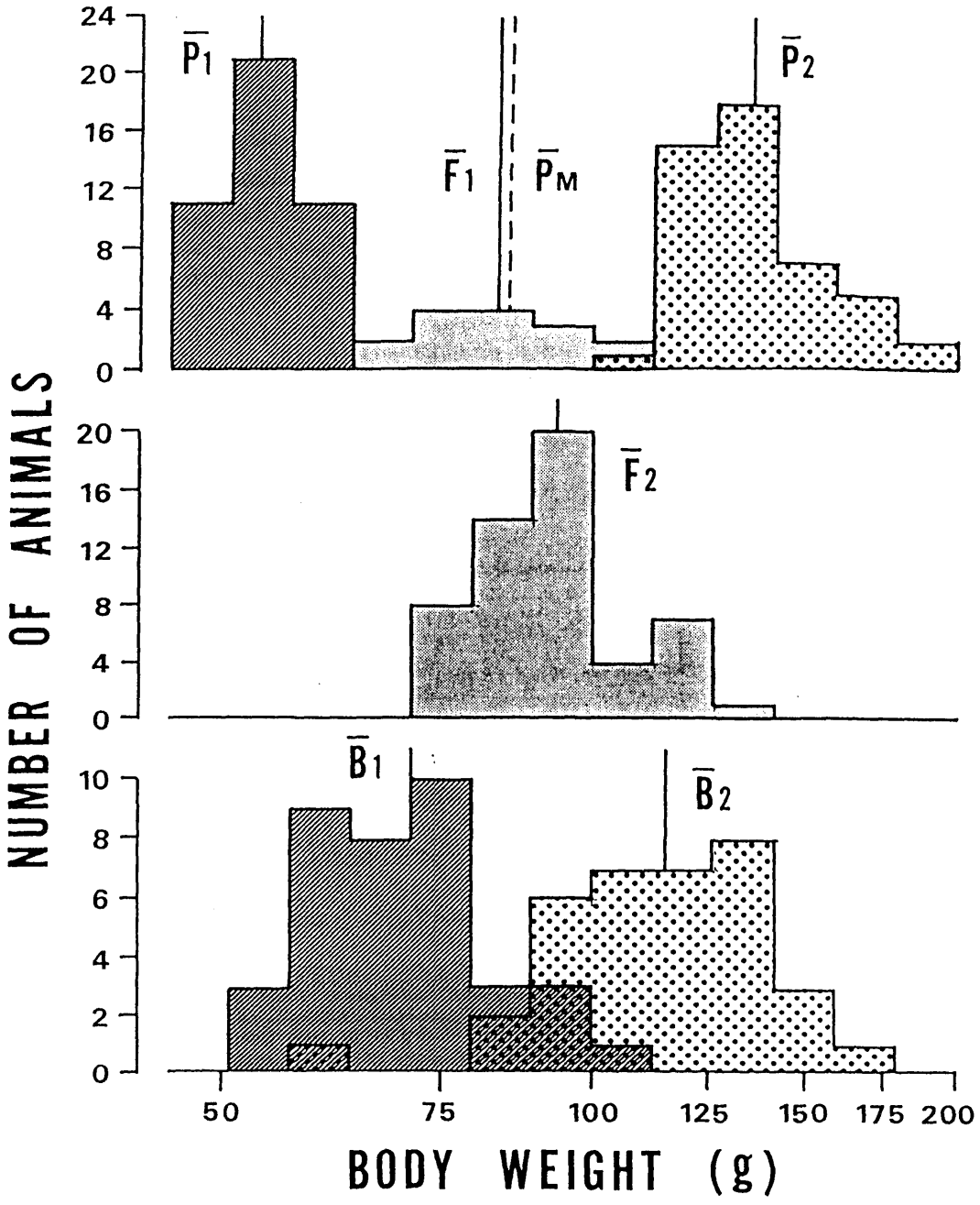

Fig. 7-1. Frequency distributions of 120-day body weight in an interstrain cross of the shrews. Vertical solid lines represent the mean body weights for individual groups, $\bar{P}_1$, $\bar{P}_2$, $\bar{F}_1$, $\bar{F}_2$, $\bar{B}_1$, and $\bar{B}_2$ (see Table 7-3 for details); broken line ($\bar{P}_M$) shows the mid-parent value. Body weights are depicted as logarithmic units to the base 10.

Chapter VIII

Kinky coat, a new autosomal recessive mutation discovered in the BAN strain

INTRODUCTION

In former chapters, unique genetic characteristics in the BAN strain itself newly developed from Bangladesh wild shrews were revealed in comparison with previously established shrew strains. The present chapter presents a morphological mutant newly discovered in the BAN strain as a fact of genetic variability within the strain.

Rexoid coat mutants have been recorded in various mammalian species, such as the house mouse (Lyon and Searl, 1989), rat (Greaves, 1981), rabbit (Castle and Nachtsheim, 1933), Guinea pig (Whiteway and Robinson, 1989), and cat (Robinson, 1982). The mutant phenotypes are inherited as recessives or dominants to normal coats. They are characterized by bent or curled vibrissae and by curved or wavy coat hairs which are shorter and/or thinner than normal.

A curly hair mutation controlled by a single autosomal recessive gene (symbol *ch*) has been found in the musk shrew (Oda, 1985b and 1989). The mutant arose in the Tr strain derived from wild shrews on Taramajima Island, Japan. The *ch*/*ch* homozygotes are fully viable and fertile. They have curly whiskers, wavy coat, and

curled long hair on the tail. These traits completely remain throughout life.

In March 1987, I found a kinky-coated female shrew in the BAN strain. Genetic analyses provided evidence for the inheritance of the kinky coat character as a single autosomal recessive gene. Phenotypically, the kinky coat mutant resembles the curly hair mutant described above in terms of the bent whiskers and curled tail hair. However, coat hair is less curved in the kinky coat mutant than in the curly hair mutant.

In the present chapter, I describe the genetic basis and morphological characteristics of the new mutation with the kinky coat character in the BAN strain of the shrews. I also test for allelism between the genes conditioning the kinky-coat and curly-hair mutant characters.

MATERIALS AND METHODS

Origin

The history of the BAN strain of the shrew was previously reported in Chapter II. The kinky coat mutant was first discovered in one young female at the time of weaning, at the fifth generation of the BAN strain. The phenotype of her single littermate could not be observed because of its death a few days after birth. The incidence of the kinky coat mutant could not be traced in other litters from the same parents,

both of which were phenotypically normal, because the male parent had been discarded before the birth of the original mutant. It is unknown from an examination of the pedigree record of the BAN strain whether the mutant gene arose spontaneously in the BAN strain or was present in the original breeding stock of the strain as a carrier.

Mating Experiments

Mating experiments were performed within the BAN strain to examine the mode of inheritance for the kinky coat. I obtained F_1 progeny from reciprocal crosses between mutant shrews and normal controls, and their subsequent F_2 and reciprocal backcross generations.

I tested for allelism of the present mutant gene with the *ch* gene previously found in the Tr strain (Oda, 1989). The BAN strain shrews, from which the kinky coat mutant was derived, were reported to be about 3 times heavier than the Tr strain shrews (Ishikawa *et al.*, 1989). Since mating success in this species can be achieved only when females are nearly as heavy as males, or lighter (Chapter IV), the large-sized, kinky-coated males in the BAN strain were crossed to the small-sized, curly-haired females in the Tr strain which were introduced into my laboratory from the Research Institute of Environmental Medicine, Nagoya University. The F_1 hybrids obtained showed a slightly unusual appearance as described later, so they

were additionally backcrossed to the kinky coat shrews.

All the crosses described above were conducted for 2 days in single-pairs. I classified the mutant character of the obtained progeny at 5 days after birth, because 1) I have found from experience that the handling of pups during the first 5 days after birth induces maternal cannibalism very frequently; and 2) the mutant shrews could be clearly distinguished from their normal littermates by their curly whiskers (Fig. 8-1). General husbandry conditions for the shrews were described elsewhere (Chapter II).

Fertility and Viability

Percent parturition, litter size, percent pup survival, and body weight were recorded on the homozygotes, heterozygotes, and homozygous normal controls employed in the above-mentioned mating experiments. The percent parturition was defined as the proportion of total number of parturitions to total number of mating trials. The litter size was determined at 5 days of age. The percent pup survival was defined as the proportion of total number of pups weaned at 20 days of age to total number of pups observed at 5 days. The body weight of young was measured at weaning.

Hair Morphology

Hair samples were obtained from 4 young and 8 adult homozygous kinky-coated shrews and, for

comparison, from 3 young and 7 adult heterozygous normal-coated animals. The young were 42 or 50 days of age and the adults were between 5 and 10 months old for both the phenotypic shrews. Coat hair was cut away from the middorsal region with safety razors; whiskers and long hair on the tail were obtained with scissors. These hair samples were air-dried and stocked at room temperature.

I observed a wide spectrum of morphological characteristics of the hair samples in three ways. First, I measured the length and maximum width of coat hair without any treatment through the use of an ocular micrometer. The hair measured was the longest in a preparation from one adult animal, because hair growth cycle has not yet been determined in this species. Second, I examined the shaft structure of coat hair under a light microscope. The coat hair samples were dehydrated and cleared in alcohol, alcohol-xylene, and xylene, followed by embedding in Canada balsam. Last, I investigated the surface texture of the shafts of coat hair, whiskers, and tail hair by means of scanning electron microscopy. The samples of these three kinds of hair were mounted on specimen stubs with double-sided adhesive tape and coated with gold in an ion sputter unit (JFC-100, Jeol, Co., Ltd., Tokyo). The samples were observed under a scanning electron microscope (JSM F-7, Jeol, Co., Ltd., Tokyo) operating

at 7 kV.

RESULTS

Genetics

The results of mating experiments, summarized in Table 8-1, agree closely with expectations based on simple autosomal recessive inheritance. The good Mendelian ratios indicate that the mutant character is of full penetrance.

An allelism test was performed between the kinky-coated males and the curly-haired females. The 6 male and 6 female F_1 progeny obtained from the 4 matings were slightly abnormal in external appearance. The tips of their whiskers were slightly bent forward toward the mouth. The long hair on the tail was slightly curved. These traits persisted in adults, but the coat hair looked normal throughout life. However, the external characteristics were usually made it difficult to distinguish the F_1 shrews from normal controls with certainty. I inferred that the characteristics appearing in the F_1 shrews were probably caused by the influence of the *ch* gene conditioning the curly hair character, because 1) such traits were reported to appear in the *+/ch* phenotype (Oda, 1989); and 2) heterozygotes for the present mutant gene could not be distinguished from homozygous normal controls by light microscopic and scanning

electron microscopic examinations of the hair, as described later. I thus classified the F_1 shrews as phenotypically normal.

To confirm precisely the independent expression of the two mutant genes, I backcrossed the F_1 females to the kinky-coated males. The 5 matings produced 13 normal and 10 mutant shrews, the segregation ratio of which was in good agreement with the expected 1:1 ratio hypothesized for the independence of the two genes (X^2-test, $0.50<P<0.70$).

From the above results, I conclude that the kinky coat character is controlled by a single autosomal recessive gene which is not allelic to the *ch* gene. Accordingly, I propose the symbol *kc* (kinky coat) for the present mutant gene found in the shrew.

Fertility and Viability

Table 8-2 shows reproductive abilities for the *kc/kc*, *+/kc*, *+/+* shrews. There were no significant differences in both percent parturition (X^2-test or Fisher's exact probability test) and litter size (t-test) among the three genotypes at $P<0.05$. Five heterozygous pups died from unknown factors at about 5 days of age, leading to significantly lower pup survival (95.1%) than for the *kc/kc* shrews (100.0%).

Table 8-3 presents details of mean body weight at weaning for the three genotypes. For males, mean body weight of the *+/kc* shrews was significantly higher than

those of the other genotypes for unknown reasons. For females, however, there were no significant differences among the three genotypes in body weight. The body weights of the three genotypes for both sexes were not significantly different from those of the +/+ control shrews (62.9 g in males and 46.6 g in females) described in Chapter V at $P<0.05$ (t-test or Cochran-Cox test).

From the above results, it is clear that the *kc* gene is not exerting any appreciable effect on fertility and viability.

External Appearance

Figure 8-1 shows gross appearances of a kinky coat homozygote and its normal littermate at 5 days of age. The homozygote had strongly curled whiskers which were readily recognizable. The colorless guard hair sparsely covering the whole body at this age was irregularly curved or crooked, and tended to be close to the body surface, compared with the straight guard hair of the normal littermate. At weaning (20 days of age), the coat hair was somewhat unkempt or untidy. The long hair on the tail was extremely wavy. These mutant traits persisted in adult shrews (Fig. 8-2).

Hair Morphology

Few studies have so far attempted to describe morphologically the coat hair of the musk shrew in detail. Under a light microscope, I first classified

the coat hair from mid-dorsum of adult heterozygous normal shrews into three distinct types (A, B, and C), according to the criteria employed in the mouse (Dry, 1926), such as the number of rows of medullary cells and the presence or absence of constrictions in the shaft. Very few intermediate types are normally found.

Type A is the overhair which has no constrictions. The thin parts of the shaft have one row of medullary cells. In the broader parts of the shaft, there may be two rows which are often very ill defined and usually become continuous because of the densely-pigmented medullary cells. The pigmentation is present in the medulla, but can be never seen in the cortex of all hair types from the BAN shrews covered with light-gray coat. By contrast, Japanese shrews with dark-gray coat generally have a medulla and cortex both of which are pigmented (my unpublished data).

Type B hair with a single constriction and Type C hair with three or two constrictions are both underhairs shorter in total length and thinner than Type A hair (Table 8-4). The hairs of both Types B and C are divided into some segments by the constrictions: the first segment is the region from the tip to the first constriction, the second segment is the region between the first and second constrictions, and so on. The first segment of Type B hair has one or two rows of medullary cells wholly pigmented, but small pigment

clumps are often found proximally. The second segment of Type B hair and all segments of Type C hair both have only one row of medullary cells completely filled with pigment granules, and they look like a ladder. Type C hair especially resembles the zigzag hair of the mouse (Dry, 1926) in light microscopic appearance.

Adult kinky-coated shrews had all of the three types of coat hair present in heterozygous shrews. For each hair type, both the length and maximum width did not differ significantly between the *kc/kc* and *+/kc* shrews at $P<0.05$ (t-test or Cochran-Cox test), as shown in Table 8-4.

Figure 8-3 shows light microscopic appearances of coat hair from adult homozygotes and heterozygotes. Two distinct kinds of abnormalities were detected for the shafts of the mutant coat.

First, the shafts of all types of the kinky coat hair were strongly waved, compared with the corresponding straight hair shafts from heterozygotes (Fig. 8-3A to F). These waves were also observed for young homozygous coat. Numerous variations were noted in both range and degree of the waviness within and among hair types from the same shrews. The regions showing waviness never co-existed with obvious abnormal structures such as irregular arrangement of the medullary cells and abnormal shaft diameter.

Second, homozygote shafts often had small

swellings with the disorganization of medullary structure (Fig. 8-3G to J). The small, different-sized swellings within and among hair types were frequently found in the first segment of Type B hair and the second or third segment of Type C hair. Apart from the hair types, the incidence of the swellings per preparation per animal averaged 5.2% (ranging from zero to 9.5%) in 8 adult homozygotes, for half of which, when they were young, the mean incidence was 2.6% (zero to 6.3%). By contrast, none of the 7 adult nor 3 young heterozygotes had such swellings in their shafts for all of the hair types.

Figure 8-4 shows characteristics of coat hair, whiskers, and long hair on the tail from homozygotes and heterozygotes under a scanning electron microscope. Three kinds of hairs from adult and young homozygotes commonly showed swellings, longitudinal fissures, twists, and hollows of the shafts. The pattern of the cuticular scales appears to be normal despite the shaft irregularity. Some of the hollows observed were conceivably marks artificially made with tweezers when I obtained the hair samples, because small cracks sometimes were found in the hollows. However, it was rare to observe heterozygotes with such artificially-made hollows in any kind of hair. It thus seems that the shafts of the homozygous hair are injured easily.

In addition to the above-mentioned abnormalities,

the coat hair from homozygotes often had flat or corrugated shaft configuration (Fig. 8-4D). The corrugation also was seen for the whiskers. A complication of some abnormalities was frequently found in shafts of all kinds of hairs. All the abnormalities observed were generally found in various parts of the shafts of the coat hair and tail hair, whereas they were frequently seen in the proximal regions of the whiskers.

The coat hair, whiskers, and tail hair of the +/*kc* and +/+ shrews were little affected in scanning electron microscopic appearance, but their coat hairs occasionally showed slight longitudinal fissures of the shafts.

It is clear that the shaft modifications discovered under the scanning electron microscope (Figure 8-4) caused waviness or curling of the shafts of the coat hair, whiskers, and tail hair seen in the gross or light microscopic appearance (Figs. 8-2 and 8-3).

DISCUSSION

The kinky coat I found is the second mutation of this kind to have been observed in the shrew. The first is the curly hair described by Oda (1989). I could not compare the hair structure between the two mutants in detail, because the microscopic examination

of the curly hair has not been performed.

The kinky coat (*kc*) gene was not observed to have any pleiotropic effects in the present study. Bennett and Greshman (1956), however, found that in many mice homozygous for the waved-1 (*wa-1*) gene, which causes curly whiskers and waved coat, the eyelids are open at birth. In the rat the zitter (*zi*) gene, which causes an ataxic disorder, is reported to produce a hair anomaly (Rehm *et al.*, 1982). The *kc* gene in the shrew should be useful as a morphological genetic marker in linkage studies, because the gene is shown to have good penetrance (Table 8-1) and to be fully fertile and viable (Tables 8-2 and 8-3). Only two visible markers, the curly hair (*ch*) gene (Oda, 1989) and the cream (*cr*) gene for coat color (Iseki *et al.*, 1984), have so far been reported in the shrew.

Laboratory rodents are well known to have a great array of rex or quasi-rex mutants, and detailed studies have been made on their mutant characters (Greaves, 1981; Lyon and Searle, 1989; Robinson, 1981; Whiteway and Robinson, 1989). The kinky coated shrew in the present report resembles the fuzzy (gene symbol *fz*) mouse both in its recessive inheritance and in the way the coat hair remains waved or curled throughout life. The shrew differs, however, in that the fuzzy coat is thin and the hair types are less readily distinguishable from each other than normal (Mann, 1964; Trigg,

1972). In addition to the *fz/fz* mice, Trigg (1972) has reported that coat hair from the soft coat (*soc*) mice is thin with occasional irregularities in diameter. He has further observed that in the hair follicles of both the two mutant mice, the dermal papillae and hair bulbs, both of which may be responsible for the normal hair growth, are short or small and rounded, respectively. In the kinky coat mutant I found, both the dermal papilla and hair bulb are considered to be little affected histologically, because 1) all three coat-hair types are easily distinguishable from each other; 2) both their length and width are nearly identical to those of normal (Table 8-4); and 3) they do not show any irregularities in the shaft diameters (Fig. 8-3A to F).

No kinky-coated shrews exhibited alopecia appearing in many rex-type mutants such as the wavy coat (Re^{WC}) in the mouse (Trigg, 1972) and the kinky (*k*) and rex (*Re*) in the rat (Robinson, 1981). Moreover, the kinky coat shrew differs greatly from the rex-type mutant mice or rats in many hair characteristics. Nevertheless, the shaft modifications such as swellings, twists, and longitudinal indentations I discovered in the present mutant (Figs. 8-3 and 8-4) are surprisingly similar to those of the wavy coat mice (Trigg, 1972). Trigg (1972) has suggested that the characteristics of the wavy coat

appear to result from a defect in the internal root sheath function of the hair follicles. Thus it is likely that in the present mutant the internal root sheath is irregular in shape, but it might be less affected than that of the wavy coat mouse, showing both variation in shaft diameter with overpigmentation of the medulla and the disruption of the normal pattern of cuticular scales (Trigg, 1972).

From the above comparisons, it is clear that the kinky coat mutant in the present study does not closely resemble any rex or quasi-rex mutants of laboratory rodents in hair structure. Histological observations will provide further information on the precise characters of the kinky coat mutant in the shrew.

Powell and Rogers (1990) have demonstrated that in transgenic mice in which a sheep wool intermediate filament keratin gene has been introduced, both the vibrisae and coat hair are wavy with some structural defects, which result from the disruption of the normal ratio of intermediate filament keratin protein to the filament-associated proteins in the hairs. Such imbalance might cause the hair anomaly observed in the present mutant, but this remains to be investigated.

SUMMARY

A kinky coat mutant was discovered at the fifth generation of the BAN strain of the shrew. Mating

experiments indicated that the kinky coat character was controlled by a single autosomal recessive gene designated *kc* (kinky coat), which was not allelic to the gene *ch* (curly hair) previously reported in the Tr strain derived from wild shrews on Taramajima Island, Japan. The *kc/kc* homozygotes were fully fertile and viable. In external appearance, adult homozygotes were characterized by curly whiskers, somewhat unkempt coat hair, and wavy long hair on the tail. Light microscopic observations showed that the shafts of the coat hair were wavy and often had small swellings with disorganization of the medullary structure. Scanning electron microscopic examinations revealed that the shafts of the whiskers, coat hair, and tail hair had abnormalities such as longitudinal fissures, twists, and hollows. These modifications apparently caused waviness or curling of the shafts of the three kinds of hairs observed.

Table 8-1. Segregation of the kinky coat (*kc*) in the shrews at 5 days after birth.

Mating ♀ X ♂	No. of matings	No. of offspring	Phenotype of offspring		Expected ratio[a]	X^2
			Normal	Kinky	Normal : Kinky	
kc/*kc* X +/+[b]	9	26	26	0	1 : 0	
+/+ X *kc*/*kc*	5	13	13	0	1 : 0	
Total	14	39	39	0	1 : 0	
kc/*kc* X +/*kc*	18	67	26	41	1 : 1	3.36^{NS}
+/*kc* X *kc*/*kc*	2	6	5	1	1 : 1	
Total	20	73	31	42	1 : 1	1.66^{NS}
+/*kc* X +/+	10	37	37	0	1 : 0	
+/+ X +/*kc*	2	9	9	0	1 : 0	
Total	12	46	46	0	1 : 0	
+/*kc* X +/*kc*	14	54	37	17	3 : 1	1.21^{NS}
kc/*kc* X *kc*/*kc*	8	29	0	29	0 : 1	

Mutant pups were identified by their curly whiskers (see Fig. 8-1).
[a]Based on simple autosomal recessive inheritance.
[b]Presumed genotype.
[NS]Not significantly different from the expected ratio at $P<0.05$.

Table 8-2. Reproductive performance in kinky-coated and normal-coated shrews.

Genotype[a]	% Parturition[b]	Litter size at 5 days after birth (n)[c]	$\bar{X} \pm SD$	Range	% Pup survival at weaning[d]
kc/kc	54.7 (35/64)	(35)	3.5 ± 1.0	2 - 6	100.0[e] (122/122)
+/*kc*	51.9 (28/54)	(28)	3.7 ± 1.4	1 - 7	95.1[f] (98/103)
+/+	70.0 (7/10)	(7)	3.1 ± 1.6	1 - 5	100.0[ef] (22/22)

[a]Genotypes of mothers employed in the mating experiments shown in Table 8-1.
[b]Proportion of total number of parturitions to total number of mating trials is indicated in parentheses.
[c]Number of litters observed.
[d]Proportion of total number of pups weaned at 20 days after birth to total number of pups observed at 5 days is given in parentheses.
[e,f]Values with the same letters are not significantly different at $P<0.05$ (Fisher's exact probability test).

Table 8-3. Body weight (g) at weaning (20 days after birth) among kinky-coated and normal-coated shrews.

Genotype[a]	n[b]	$\bar{X}$ ± *SD*	Range
Male			
kc/kc	38	56.2 ± 10.0[d]	30.8 - 76.1
+/*kc*	24	63.8 ± 11.3[e]	36.6 - 80.1
+/_[c]	37	57.8 ± 9.2[d]	42.0 - 74.7
Female			
kc/kc	49	47.9 ± 7.7	23.8 - 64.3
+/*kc*	37	49.7 ± 7.1	33.6 - 62.9
+/_[c]	38	48.6 ± 6.5	36.3 - 61.5

[a]Genotypes of progeny produced from the mating experiments shown in Table 8-1.
[b]Number of shrews weighed.
[c]+/*kc* or +/+.
[d,e]Means with the same letters are not significantly different at $P<0.05$ (t-test) for males; there are no significant differences between any genotypes for females.

Table 8-4. Measurements ($\bar{X} \pm SD$) of three coat-hair types (*A*, *B*, and *C*) from adult kinky-coated (*kc/kc*) and normal-coated (+/*kc*) shrews.

Hair type[a]	Segment of hair[b]	Length (mm)		Maximum width (μm)	
		kc/kc	+/*kc*	*kc/kc*	+/*kc*
A	1st	(8)[c] 5.9 ± 0.7	(5) 7.6 ± 1.8	(8) 49 ± 2	(5) 50 ± 3
B	1st	(8) 3.1 ± 0.4	(6) 2.9 ± 0.6	(8) 41 ± 3	(6) 38 ± 3
	2nd	(8) 1.4 ± 0.3	(6) 1.3 ± 0.5	(8) 19 ± 5	(6) 18 ± 4
C	1st	(6) 0.9 ± 0.1	(5) 0.9 ± 0.1	(6) 11 ± 2	(5) 12 ± 3
	2nd	(6) 1.1 ± 0.1	(5) 1.2 ± 0.1	(6) 20 ± 0	(5) 20 ± 0
	3rd	(6) 0.9 ± 0.1	(5) 1.0 ± 0.0	(6) 15 ± 0	(5) 17 ± 3
	4th	(6) 0.8 ± 0.2	(5) 0.6 ± 0.3	(6) 9 ± 4	(5) 12 ± 2

[a]Classified according to the criteria described by Dry (1926). Characteristics of the three hair types are described in the text.
[b]Each hair type was divided into some segments by constrictions in the hair.
[c]Number of shrews examined.

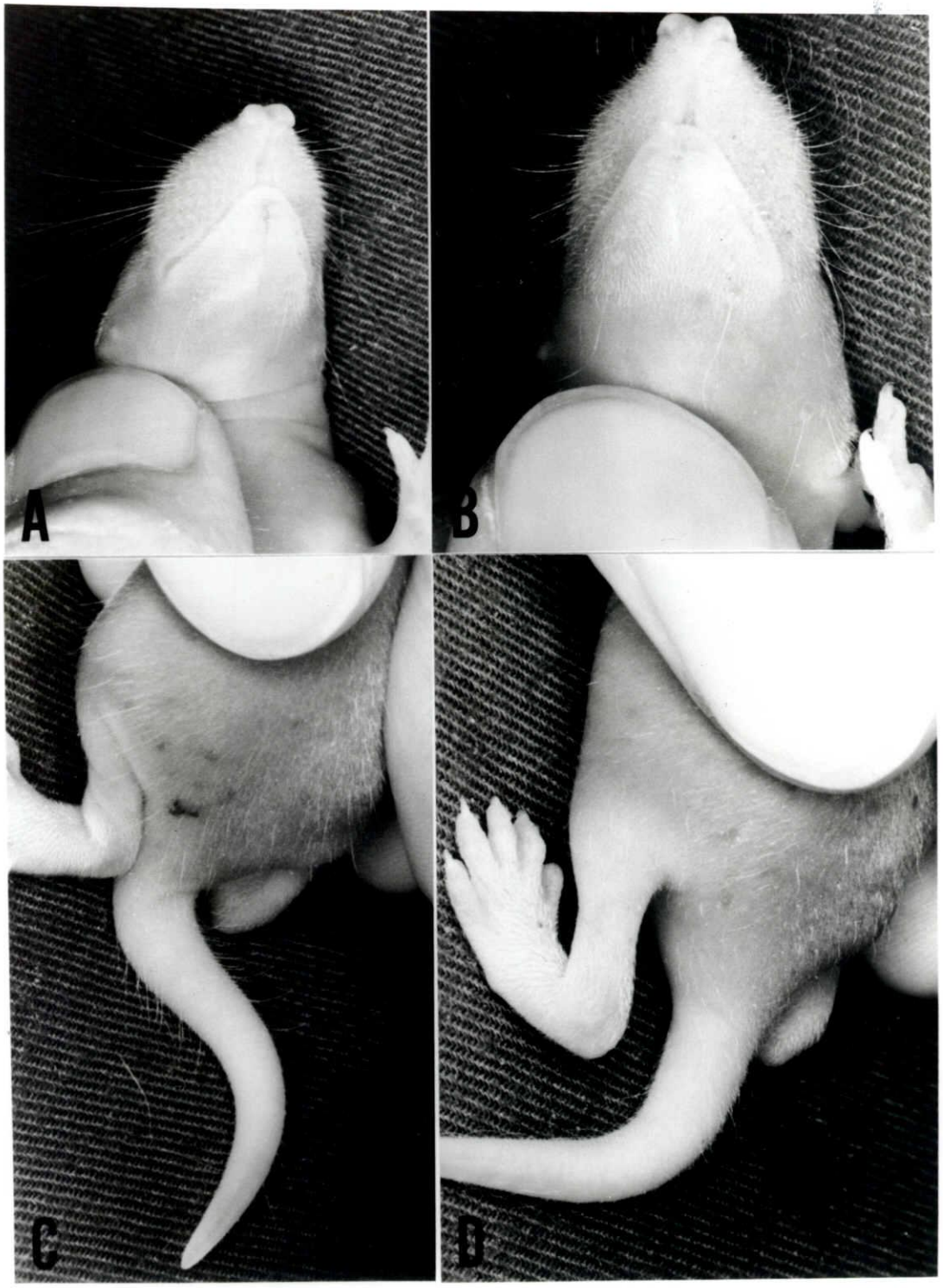

Fig. 8-1. A normal-coated shrew (*A* and *C*) and the kinky-coated littermate (*B* and *D*) at 5 days of age. Before growing a kinky coat, the mutant pup is readily distinguishable from the normal sib by the curly whiskers.

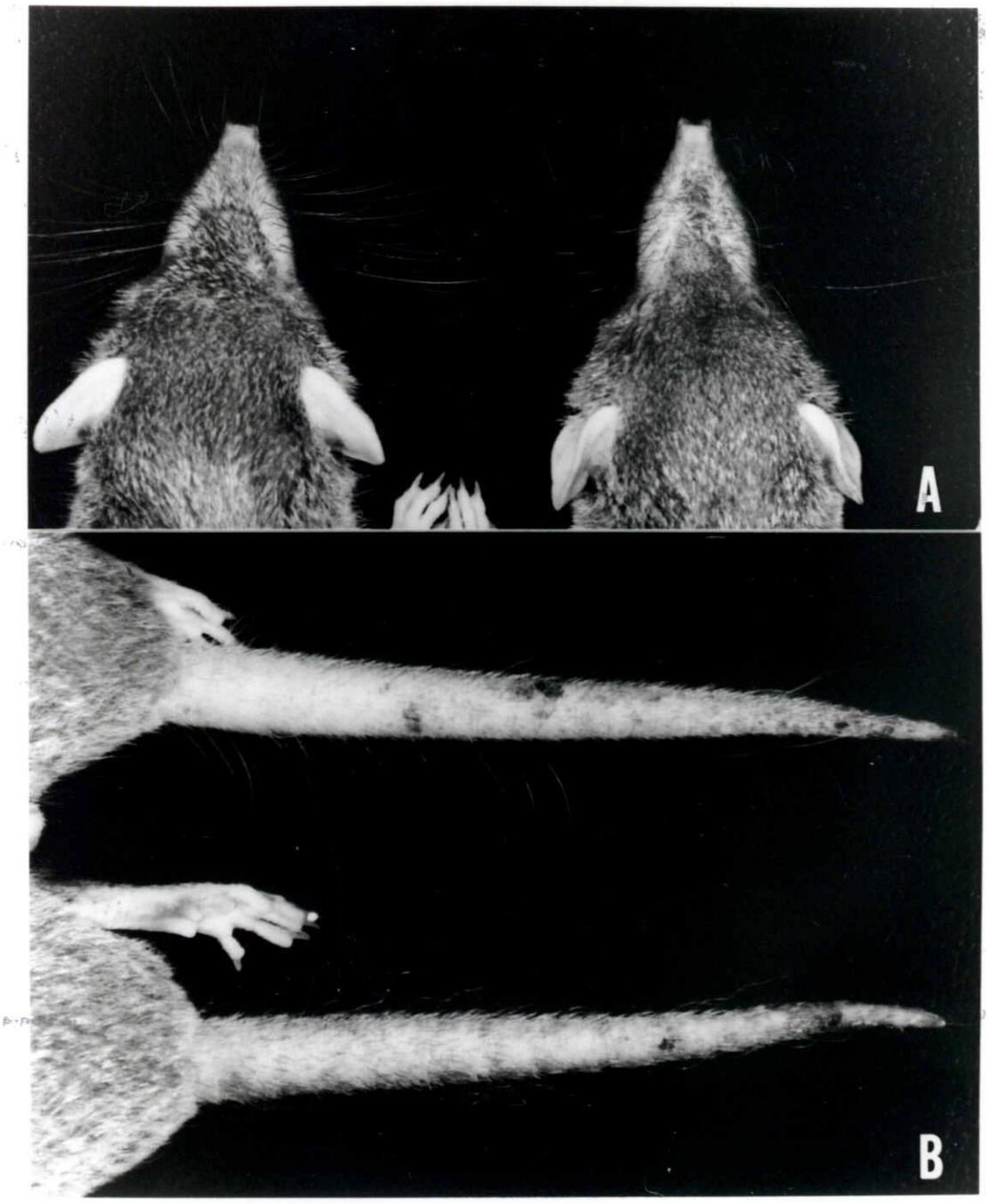

Fig. 8-2. (*A*) Straight whiskers of a heterozygous normal-coated shrew about 11 months old (left) and curled whiskers of a homozygous kinky-coated shrew about 7 months old (right). (*B*) Straight long hair on the tail of the +/*kc* shrew (top), contrast with wavy long hair of the *kc*/*kc* shrew (bottom).

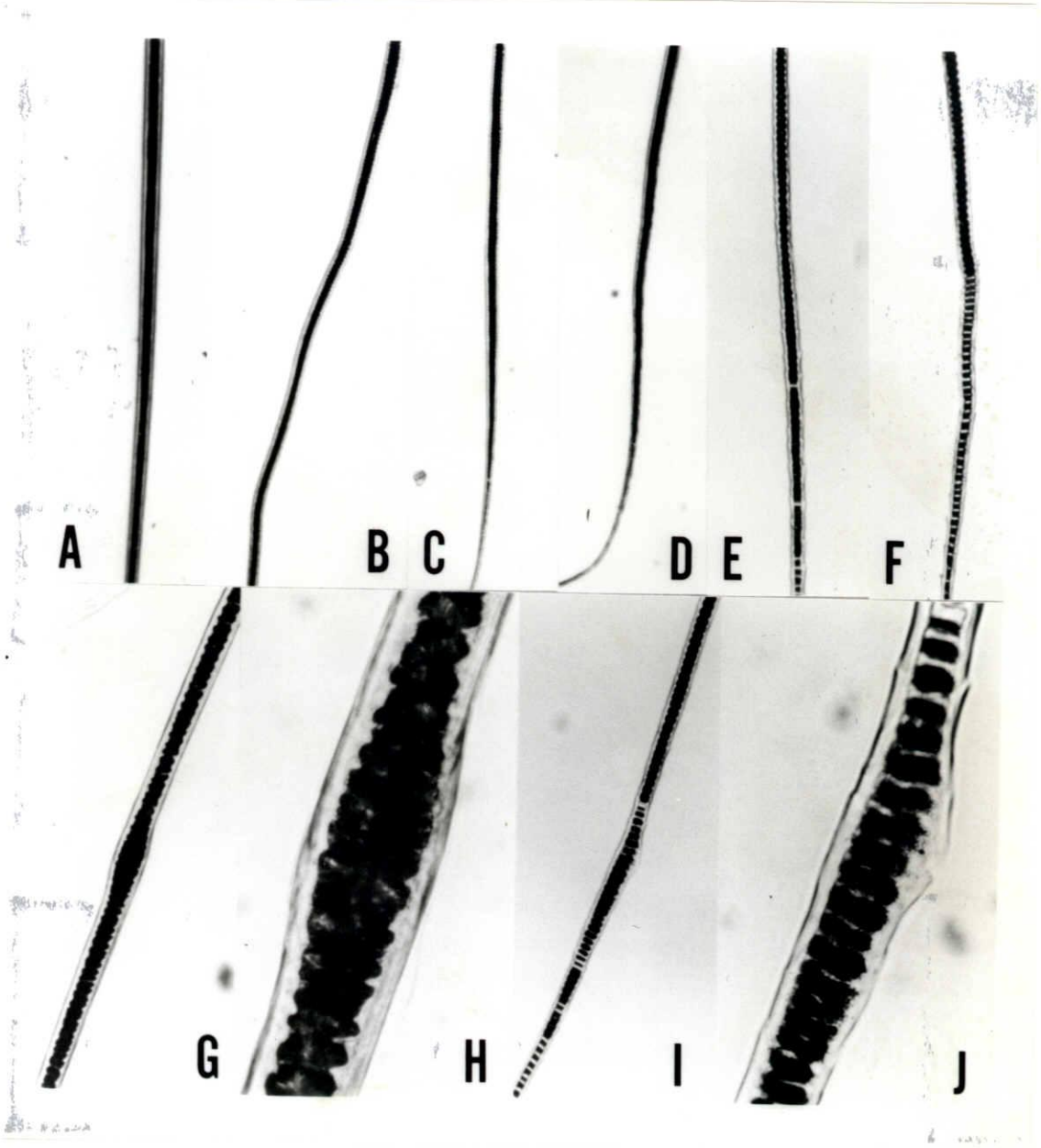

Fig. 8-3. Light micrographs of coat hair from the +/*kc* and *kc*/*kc* shrews. (*A*) Proximal region of a +/*kc* Type A hair at about 10 months of age (X32). (*B*) Proximal region of a *kc*/*kc* Type A hair at about 6 months (X32). (*C*) Proximal region of first segment of a +/*kc* Type B hair from the same shrew as in (*A*) (X32). (*D*) Proximal region of first segment of a *kc*/*kc* Type B hair from the same shrew as in (*B*); narrow base of the shaft shows a constriction (X32). (*E*) Middle region of second segment of a +/*kc* Type C hair from the same shrew as in (*A*) (X80). (*F*) Middle region of second segment of a *kc*/*kc* Type C hair at about 5 months (X80). (*G*) Shaft swelling with a breakdown of medullary structure on first segment of a *kc*/*kc* Type B hair at about 6 months (X80). (*H*) High magnification of (*G*) (X320). (*I*) Shaft bulging with the disorganization of medullary structure on second segment of a *kc*/*kc* Type C hair at about 7 months (X80). (*J*) High magnification of (*I*) (X320). See text for explanation of characteristics of the three coat hair types.

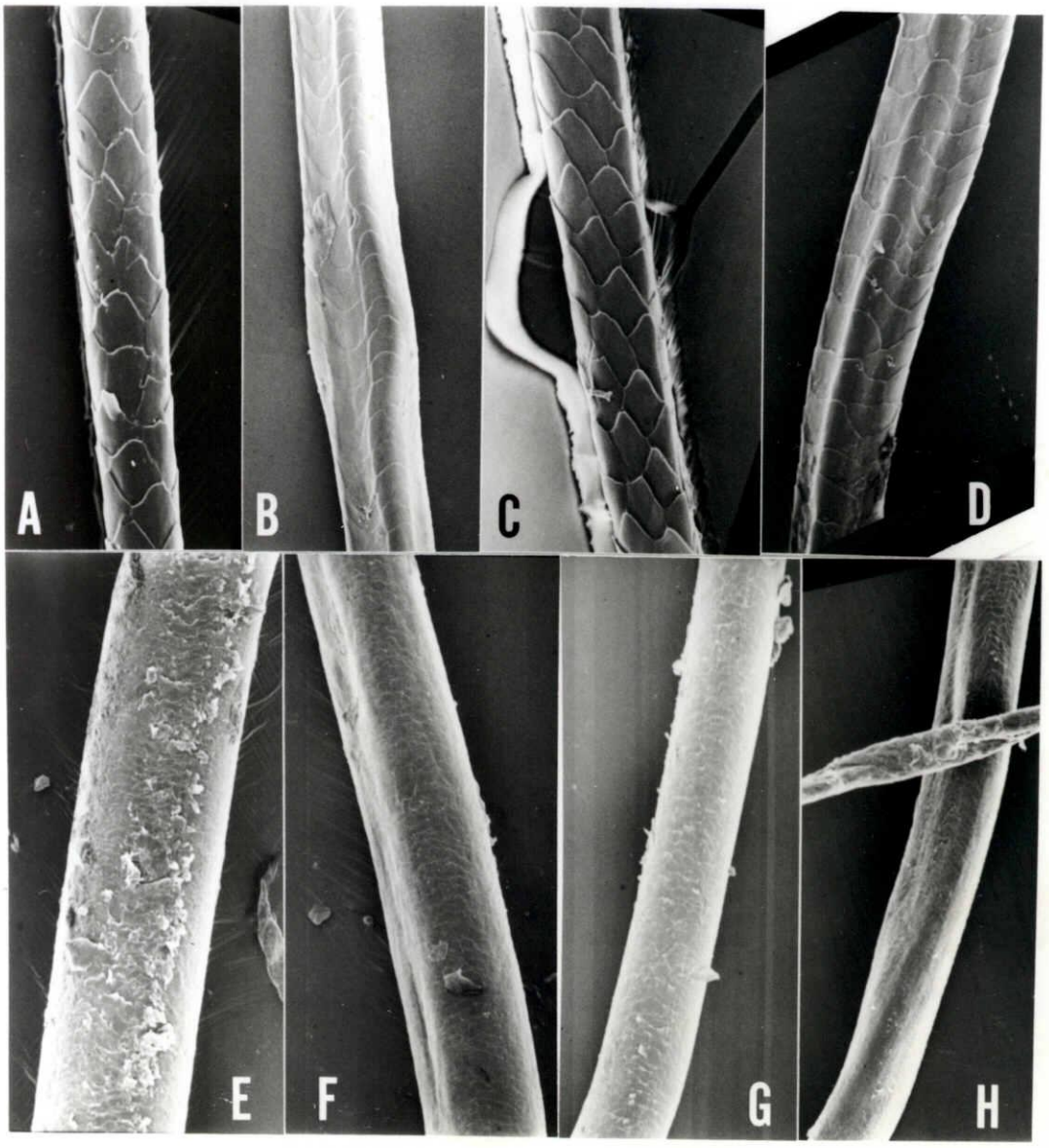

Fig. 8-4. Scanning electron micrographs of coat hair, whiskers, and long hair on the tail from the +/*kc* and *kc*/*kc* shrews. (*A*) Middle region of a +/*kc* Type A coat hair at about 6 months of age (X500). (*B*) Middle region of a *kc*/*kc* Type A coat hair at 50 days (X500), showing a longitudinal fissure and swelling of the shaft. (*C*) Proximal region of first segment of a +/*kc* Type B coat hair at about 6 months (X500). (*D*) Proximal region of first segment of a *kc*/*kc* Type B coat hair at about 5 months (X500), exhibiting a slight twisting and corrugation of the shaft. (*E*) Proximal region of a +/*kc* whisker from the same shrew as in (*A*) (X300). (*F*) Proximal region of a *kc*/*kc* whisker from the 6-month-old shrew used in (*B*) (X300), showing a longitudinal indentation or hollows. (*G*) Proximal region of a +/*kc* long hair on the tail from the same shrew as in (*A*) (X300). (*H*) Proximal region of a *kc*/*kc* long hair on the tail from the same shrew as in (*F*) (X300), having a twist and hollows.

Chapter IX

General discussion

The musk shrew has been domesticated as a unique insectivore laboratory animal species since the early 1970s (Kondo, 1985a; Oda and Kondo, 1977). Most of the shrew strains have been established from wild populations trapped in Japan (Iseki, 1985; Namikawa *et al.*, 1985; Oda, 1985a; Oda and Kondo, 1976; Oda and Shigehara, 1978). However, wild shrews are widely distributed throughout tropical Asia and the Far East (Oda, 1985a), and differ geographically in their genetic characteristics, *e.g.*, body size, coat color, chromosome number (Ishikawa *et al.*, 1989), and mtDNA type (Yamagata *et al.*, 1990).

Recent two genetical studies on wild shrews captured in Bangladesh noted their unique genetic properties as follows. First, the Bangladesh shrew population has three types of alleles at the *Amy-1* locus, one of which is never found in wild populations from Japan, Indonesia, and Sri Lanka (Tsubota and Namikawa, 1988). Second, the Bangladesh population is genetically differentiated from the Japanese, Indonesian, and Sri Lanka populations to the extent which can be compared to intersubspecific differences in *Mus musculus* based on mtDNA types (Yamagata *et al.*, 1990). In Chapter II, the Bangladesh shrew was

morphologically characterized by one of the largest animals in this species and by the light-gray pelage, compared with the dark-gray coat of small Japanese shrews. These facts provide the necessity that the Bangladesh wild shrew should be bred as a new laboratory shrew. Hence I have successfully developed the BAN strain originating from 4 male and 7 female wild shrews in Bangladesh (Chapter II). The present thesis revealed similarities or differences in genetic characteristics between the BAN strain and previously established shrew strains and disclosed genetic variability within the BAN strain.

The chromosome analysis using the conventional Giemsa staining method in Chapter III indicated that the shrew strains examined can be divided into two types on the basis of chromosome number: the 2N=30 type and the 2N=40 type. The former was only found in the SRI strain originating from a wild population in Sri Lanka. The latter was composed of the BAN strain and Japanese strains, NAG and OKI, respectively derived from Nagasaki and Okinawa Island. With the exception of the Y chromosomes, no clear differences were demonstrated among the 2N=40 karyotypes of the three strains. This is only one point of genetic similarity in the BAN and the other strains, discovered in the present thesis. The Y chromosomes differed extremely in both size and morphology: a large submetacentric in

the BAN strain, a small metacentric in the Japanese strains, and a small submetacentric in the SRI strain. Thus the Y chromosome is very useful as a marker chromosome in the laboratory shrew.

In Chapter IV, I performed mating tests between the BAN and NAG strains because of the early-mentioned extreme genetic relationship in their wild shrew populations, from which the two strains were derived. This provided a very much surprising result that fertile and viable F_1 hybrids could be obtained by crossing the small NAG females with the large BAN males, whereas in the reciprocal cross of the large BAN females and the small NAG males no pregnant BAN females were found, which seemed to be due to highly aggressive fighting behaviors of the large BAN females never allowing the small NAG males to mount and copulate. Various types of crosses using F_1 and F_2 progenies obtained by the former cross and the two parental shrews proved that mating success in the induced ovulatory shrews is achieved only in a condition that females are nearly as heavy as males, or lighter. I therefore believed that the BAN and NAG strains originating from different geographical races have evolved a partial premating isolation mechanism with concomitant body size differentiation. Such a reproductive isolation mechanism has not yet been reported in this species. Because the BAN shrew is one of the largest animals in

this species (Chapter II), the shrew might be in the process of speciation at the present time.

The above showed the great importance for the study on the body size difference of the BAN and NAG strains. The 120-day adult body weight of the BAN strain was 135.3 g in males and 82.0 g in females, compared with 52.9 g and 34.2 g, respectively, in NAG males and females. Why does such a great weight difference exist in the two strains? To resolve this question from a genetical view point, I initially examined postnatal growth and development in the two extreme strains (Chapter V). Timing of postnatal appearances of 13 characters, such as eye open, ear open, and dorsal pigmentation, was nearly identical in the two strains despite their body weight difference. The growth curve, which was composed of linear and decaying exponential functions, provided the best description of the growth data in the shrews of both the strains, because their body weights increased at relatively constant rates for several weeks after birth and subsequent growth rates rapidly decreased. This means that the shrew growth curve has no inflection point, which is a very unique form because sigmoid growth curves with an inflection point are generally adopted for laboratory mice and rats (Eisen, 1976; Gall and Kyle, 1968). The growth analysis of the BAN and NAG strains revealed that the difference in the 120-day

adult body weight of the two strains reflects both an approximately 2.5 times longer duration of linear growth phase and an approximately 1.5 times higher growth rate during this phase in the BAN strain.

To confirm whether the above-mentioned two growth characters were under genetic controls, I analyzed the growth pattern of the F_1 progeny obtained from the cross between the large BAN males and the small NAG females (Chapter VI). This indicated that both the duration of linear growth phase and the growth rate during this phase in the F_1 shrews were approximately intermediate between the corresponding values of the parental strains. This means that the two growth characters appear to be genetic components, like growth curve parameters of laboratory mice (Eisen, 1974 and 1976), important in understanding the genetics of postnatal growth in the shrews. Direct genetic effects on the growth characters await evaluation with regard to this matter.

In Chapter VII, the distribution of 120-day adult body weight in the F_1, F_2, and reciprocal backcross progenies produced from the cross between the small NAG females and the large BAN males gave the evidence that the weight difference between the two strains is under the polygenic control. The analysis of the adult body weight of the two parental shrews and their hybrid generations based on statistical techniques of quanti-

tative genetics revealed that a minimum of 8 freely segregating genes contribute to the weight difference of the extreme shrew strains. This estimate can be interpreted as average properties of a group of "quantitative trait loci" actually influencing the body weight difference of the two strains, as Paterson *et al*. (1991) have pointed out. The gene number estimated is thus considered to be fundamental for the study of mechanisms of heredity and evolution in the adult body weight of the shrews.

From the succeeding growth studies described above, I believe that the BAN shrew, one of the largest animals, palys a major role in understanding the process of body size differentiation in this species.

The above-mentioned succeeding studies have been revealing the very unique genetic characteristics of the BAN strain itself. Now I focus on describing the genetic variability within the strain. In Chapter VIII, a kinky coat mutant controlled by a single autosomal recessive gene (symbol *kc*) was presented. This is the first morphological mutant discovered in the BAN strain. Currently I have discovered the second morphological mutation causing the eye-ball defect at the 14th generation of the strain. Both parents of the original mutant have phenotypically normal eyes and at the present time they produced a total of 8 normals and 3 mutants (one male and two females) with the defect of

both or either eyes, the segregation ratio of which was likely to agree with the expected 3:1 ratio based on simple autosomal recessive inheritance. I am now precisely investigating the genetics and phenotype of the mutant character. Although two visible mutants, the curly hair (Oda, 1989) and cream coat-color (Iseki *et al.*, 1984), have been reported in this species so far, there are no cases that more than one mutant are discovered within the same strain which was derived from the small number of wild shrews. In addition, Yamagata *et al.* (1987) reported that within the BAN strain there are two types of mtDNAs, which are estimated to be genetically differentiated from each other to the level that can be compared to mice-intersubspecific differences. They also reported that such a case is not present in Japanese, SRI, Bog (derived from Indonesian wild shrews) strains. I have been conserving the unique two mtDNA types in the BAN strain. These facts may show higher genetic heterogeneity among the individuals of the BAN strain than those of the other strains.

From the above findings, it is clearly concluded that the BAN strain newly established from wild shrews in Bangladesh is characterized by the tremendous genetic potential for improvement in the genetic knowledge of the laboratory shrew. For example, I proved that fertile and viable F_1 hybrids can be obtained by

crossing the BAN males with the NAG females, despite their great genetic differences (Chapter IV). By making use of the hybrids obtained, it may be feasible to identify new biochemical genetic markers and molecular markers and to construct their linkage maps. Such basal genetic data have not yet accumulated for the shrews. Moreover the BAN shrew must be very useful for studies on speciation and morphological differentiation in this species.

Chapter X

General summary

I have succeeded in developing a new strain, BAN, derived from wild musk shrews in Bangladesh. I described a historical account of the origin of the BAN strain and examined its genetic characteristics with special reference to chromosomes, reproductive isolation mechanism, postnatal growth, and a morphological mutant.

Forty-nine male and 49 female wild shrews were collected in the campus of Bangladesh Agricultural University from October through November in 1983 and from December in 1985 to January in 1986. The shrews captured were of various ages and had light-gray coats. Except for the 12 shrews introduced into my laboratory to establish the BAN strain, the total length and body weight ranged from 17.2 to 31.9 cm and from 32.5 to 147.0 g in males and from 21.1 to 26.6 cm and from 40.8 to 110.0 g in females. Pregnant females were found throughout the trapping period, and the average fetal litter size was 3.54. Five males and 7 females of the shrews captured in 1983 were transported to my laboratory. After being reared more than 100 days in the laboratory, their total length and body weight averaged 27.6 cm and 147.3 g in males and 24.6 cm and 81.7 g in females. This showed that the Bangladesh

shrew is one of the largest animals in this species. The shrews introduced (except for one male) produced a total of 59 offspring, which were regarded as the first generation of the BAN strain. The gestation period and average litter size were between 28 and 30 days and 3.47, respectively. The BAN strain has been maintained as a closed breeding colony consisting of about 60 individuals at each generation.

The chromosome analysis using the conventional Giemsa staining method revealed that the chromosome number of the BAN shrew was 2N=40, being exactly the same as in the shrews of NAG (derived from Nagasaki, Japan) and OKI (from Okinawa Island, Japan) strains, whereas that of the SRI strain from Sri Lanka was 2N=30. With the exception of the sex chromosomes, no clear differences were demonstrated among the 2N=40 karyotypes of the BAN and Japanese strains. The X chromosomes of the BAN, Japanese, and SRI strains were all large metacentrics. On the contrary, the Y chromosome was a large submetacentric in the BAN strain, a small metacentric in the Japanese strains, and a small submetacentric in the SRI strain. The Y chromosome is thus very useful as a marker chromosome in the laboratory shrew.

Mating tests between the large BAN and small NAG shrews were performed. In the 16 mating trials between 12 BAN females (mean body weight of 87.9 g) and 11 NAG

males (52.3 g), highly aggressive fighting behaviors of the BAN females toward the NAG males were observed. The BAN females stayed in nest boxes in cages, whereas the NAG males kept out during the mating trails. Pregnant females, diagnosed by palpation at about 16 days after the separation of the pairs, were never found in the trials. On the contrary, in 6 of the 11 trails between 8 NAG females (34.2 g) and 6 BAN males (145.9 g), viable and fertile F_1 hybrids (8 males and 8 females) were produced. The F_1, subsequent F_2 and reciprocal backcross progenies appeared neither external nor behavioral abnormalities. Various types of crosses using the F_1, F_2, and the two parental shrews showed that mating success was only in a condition that females were nearly as heavy as males, or lighter. Although body weights of musk shrews from different geographical areas were reported to vary from 43.5 to 147.3 g in males and from 26.0 to 82.0 g in females, males in the respective areas were constantly about 1.7 times heavier than the corresponding females. These results therefore suggest that the difference in body weight between sexes paired greatly affect mating success in the cross between the two strains of induced ovulatory shrews, which is believed to be a partial premating isolation mechanism.

Postnatal growth and development were studied in the BAN and NAG strains. Mean body weight at 120 days

after birth in the BAN strain was 135.3 g in males and 82.0 g in females, compared with 52.9 g and 34.2 g, respectively, in NAG males and females. Timing of postnatal appearances of 13 characters, such as eye open and ear open, was nearly identical in the two strains. A growth equation composed of linear and decaying exponential functions was fitted to the growth data for the shrews. The period from birth to the day when the shrew reached a body weight plateau could be divided into two phases: a linear growth phase followed by a period of gradually declining growth rate. The growth phase was about 60 days long in males and about 40 days in females of both strains. However, the BAN strain exhibited linear growth for 34 days in males and for 25 days in females, whereas the NAG males and females did so for only 15 and 10 days, respectively. Growth rates during the linear growth phase were 3.0 g/day in males and 2.1 g/day in females for the BAN strain, while for the NAG strain the respective rates were 1.7 and 1.6 g/day. Consequently, the difference in 120-day adult body weight between the two strains reflects both the longer duration of the linear growth phase and the higher growth rate during this phase in the BAN strain.

The growth trajectories of both sexes of the F_1 hybrids obtained by crossing the small NAG females with the large BAN males were situated roughly midway

between those of the two parental strains throughout the entire growth process. For each sex, the duration of the growth phase in the F_1 shrews was nearly identical with that of the two parental strains. The duration of the linear growth phase of the F_1 hybrids (29.8 days long in males and 19.0 days in females), however, was approximately intermediate between those of the parental strains. Likewise, growth rates during the linear growth phase of the F_1 shrews (males 2.1 g/day and females 1.8 g/day) were roughly intermediate between those of the parental strains. It is clear that both the duration of linear growth phase and the growth rate during this phase reflect genetic differences in the growth patterns of the shrews.

Mean 120-day adult body weights of the F_1 and F_2 generations obtained were approximately intermediate between those of the two parental strains. The means of reciprocal backcross generations were approximately halfway between the respective parents. The distribution of the body weight was unimodal in the segregating generations, but a small, extra peak was observed in the F_2 shrews. These provided the evidence that the weight difference between the two parental strains was under the polygenic control. The biometrical analysis of the weight data on the simple logarithmic scale indicated that a minimum of 8 freely segregating genes contributed to the difference in the adult body weight

of the extreme parental strains. Although the gene number calculated was considered to be a rough estimate because of 1) unequal contribution of environmental variance caused by both maternal effects and litter sizes in the two parental and hybrid generations; and 2) small sample sizes of the hybrid groups, it provided fundamental information about mapping individual polygenes controlling the adult body weight of the shrews.

A kinky coat mutant was discovered at the fifth generation of the BAN strain and was controlled by a single autosomal recessive gene (symbol *kc*), which was not allelic to the gene *ch* (curly hair) previously reported in the Tr strain derived from wild shrews on Taramajima Island, Japan. Since the *kc/kc* homozygotes were fully fertile and viable, the *kc* gene should be useful as a visible genetic marker in linkage studies. In external appearance, adult homozygotes were characterized by curly whiskers, somewhat unkempt coat hair, and wavy long hair on the tail. Light microscopic observations showed that the shafts of the coat hair were wavy and often had small swellings with disorganization of the medullary structure. Scanning electron microscopic examinations revealed that the shafts of the whiskers, coat hair, and tail hair had abnormalities such as longitudinal fissures, twists, and hollows. These modifications apparently cause waviness or curling of the shafts of the three kinds of hairs

observed.

From the above findings, it is clear that the BAN strain newly established from wild shrews in Bangladesh is characterized by its unique genetic potential for improvement in the basal genetic knowledge of the laboratory shrew.

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