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Population Ecology of the Japanese Field Vole (Microtus montebelli) in Kyushu

IV. Criteria for Determining Breeding Condition and Breeding Season

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The correspondence between the external characters commonly used for determining fecundity of the Japanese field vole, Microtus montebelli, in the field and the presence or absence of testicular and caudal epididymal sperm or histological observations of the female reproductive organ was established. The male breeding condition was grouped into the three types (M-I, M-II and M-III) by the relationship between the testis size and the presence or absence of sperm in both the testis and the caudal epididymis; since males with a visible scrotal bulge had testes of 10 mm or more in length and always belonged to the M-III, such a scrotum is regarded as a single criterion for the reproductive activity of the live males in the field. The female breeding condition also was sorted into the three types (F-I, F-II and F-III) by the uterine situation and whether placental scars and corpora lutea are visible to the naked eye; the teats which are sucked by young, perforated vulva and pregnancy detected by palpation (only for the individuals having embryos more than 25 somites) are utilized as useful criteria to determine the reproductive activity of the live females. Basing on the above criteria, the breeding season at Mt. Hitome was fundamentally spring and fall.

INTRODUCTION

Many papers have been published on the reproductive activity of the Japanese field vole, *Microtus montebelli*, in the field (Watanabe, 1962; Miyao *et al.*, 1966; Seki *et al.*, 1966; Shiraishi, 1967; Kanamori and Tanaka, 1968; Abe, 1974; Kaneko, 1976; Kimura *et al.*, 1980). The vole's breeding condition has been generally judged by the size of the testes and/or visibility of the caudal epididymal tubules in the males, and by the perforation or non-perforation of the vulva, conspicuousness or inconspicuousness of the teats, size of the uterus and presence or absence of embryos in the females. For the males, however, only a few authors really have ascertained the presence of sperm in the testes and/or caudal epididymides, and for the females nobody has defined the relationship between the external characteristics caused by the reproductive activity involving early development and histological observations of the reproductive organ. On the other hand, studies of the mechanism of reproduc-

tion including oestrus cycle and ovulation of *Microtus* species in captivity have been extensive from the viewpoint of domestication for supplying them as experimental animals (Greenwald, 1956; Breed, 1967; Kirkpatrick and Valentine, 1970; Cross, 1972; Gray et al., 1974; Milligan, 1975; Goto et al., 1977; Charlton et al., 1978; Goto and Hashizume, 1978, etc.). It, however, is difficult to apply directly these results as criteria of the breeding condition of the vole in the field.

The aim of this study is to make clear the correspondence between the external characters commonly used in the field for determining the breeding condition and the presence or absence of sperm or histological observations of the female reproductive organ, and also to know more exactly the breeding condition in the field. It is another aim to clarify the breeding season of the vole on the basis of these results.

MATERIALS AND METHODS

One hundred and forty-seven voles (74 males and 73 females) were used for confirming the presence or absence of sperm and histological observations of the female reproductive organ, which were all obtained at Mt. Hitome (altitude of 1,287 m) locating on the border between Kumamoto and Ōita Prefectures in northern Kyushu monthly from December 1975 to November 1976 except January and July. The following data, i.e. the date, body weight, head and body length, tail length, hind foot length apd ear length were recorded. Especially, for the males having descended testes, the length of the scrotum also measured over the pelage. After autopsy, the details of the male breeding condition were noted about the size of the testis and whether the caudal epididymal tubules were visible to the naked eye by the method of Jameson (1947, 1950). The females were examined about the conspicuousness of the teats and vaginal perforation before autopsy, and the size of the uterus, presence or absence of the corpora lutea, embryos and placental scars after autopsy. These voles were preserved in 10% neutral formalin.

In the laboratory, the presence or absence of sperm in the testis and caudal epididymis was confirmed mainly by the smear method and partially by the histologically routine method. The reproductive organ of all females was histologically examined. Furthermore, the sum total of 180 adults (96 males and 84 females) obtained from December 1975 to November 1978 were used for discussing the breeding season of the vole.

RESULTS

Breeding condition of the males

1) Types of the breeding condition by the smear method

Since the correlation coefficient between the head and body length or body weight increasing with growth and the testis length was 0.562 or 0.696 respectively, a mutual relation was hardly found. The significant correlation was not recognized (r=0.669) either between the scrotum length and the

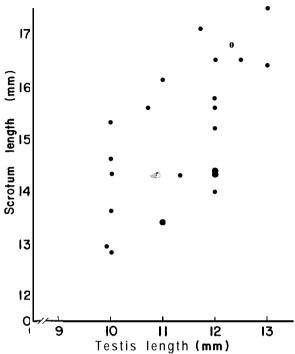


Fig. 1. Relationship between the testis and the scrotum length in *Microtus montebelli*. , sperm presence; , sperm absence.

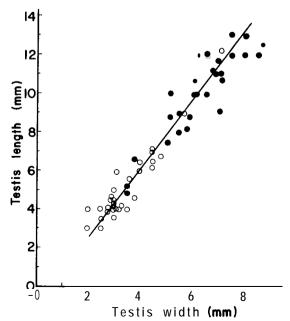


Fig. 2. Relationship among the testis length, its width and the presence or absence of sperm. ●, sperm presence; ○, sperm absence.

testis length. In the individuals with a bulge of the scrotum, the testis was about 10 mm or more in length, and both the testis and the caudal epididymis had sperm except only one old vole (52.0 g) (Fig. 1). There were, however, a considerable number of males with sperm also within the individuals devoid of the scrotal bulge.

The relationship among the testis length and its width, and the presence or absence of sperm is shown in Fig. 2. As the result, the correlation between the testis length and width was confirmed with a significance (r=

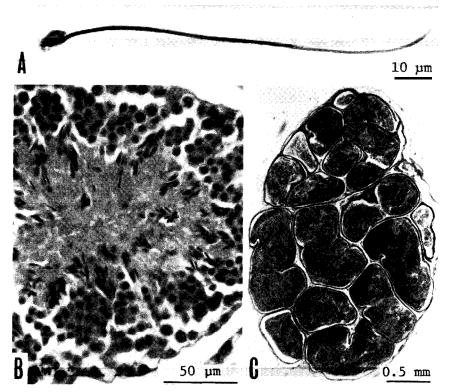


Fig. 3. Photomicrographs showing the male reproductive activity (M-III). A, an epididymal spermatozoon with the hooked head in smear; B, cross section of a seminiferous tubule at the stage of spermatogenesis; C, cross section of the caudal epididymis with sperm in the tubules.

Table 1. Types of the male breeding condition and the presence or absence of sperm in *Microtus montebelli* collected from December 1975 to November 1976.

Sperm	Туре				
	M-I	M-II	M-III		
Presence Absence	0 24	10 1'2	22 1		

0.971, p<0.001); the testis of 5x3 mm and less in length and width had no sperm, and one over 5×3 mm to 9 x6 mm either contained sperm or had no sperm. In the individuals with the testis larger than 9x6 mm, sperm were seen in both the testis and the caudal epididymis (Fig. 3) except the old male mentioned above. Thus, the male breeding condition was divided into the three types on the basis of the value of the length x the width of the testis (hereinafter referred to as the testicular value), i.e. M-I (the first type of the male) 115, 15<M-II \le 55 and M-III \ge 55.

The 74 males were grouped by this method and the relation between the type and the presence or absence of sperm was examined (Table 1). All of the individuals belonging to the M-I were devoid of sperm in both the testis and the caudal epididymis; the M-II individuals consisted of some with sperm and some lacking sperm, and a minimum testicular value was 17.2 for the males with sperm and a maximum one was 51.3 for the males devoid of sperm; the M-III ones had sperm except a male. In 6 males (60%) out of the M-II ones with sperm and in all the M-III ones, the caudal epididymal tubules could be distinctly seen with the naked eye.

2) Relationship between the breeding condition and the body weight

Fig. 4 shows the relation among the testicular value, presence or absence of sperm and body weight. In the M–I individuals (N = 24), a maximum weight was 35. Og and the individuals less than 25 g in weight constituted 75.0 % of them. In the M-II voles, a minimum weight of those lacking sperm was 21.7 g (testicular value of 17. 1) and a maximum was 49.3 g (32.6), while a minimum weight of males with sperm was 22.0 g (25.5) and a maximum was 48.2 g (17.2). All the M-III voles was 29. 1 g and over in weight.

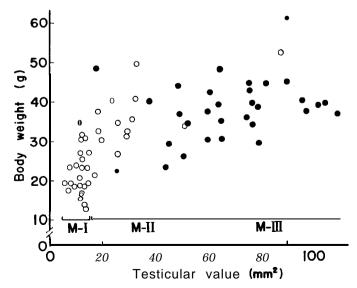


Fig. 4. Relationship among the body weight, testicular value and the presence or absence of sperm. , sperm presence; , sperm absence.

On the other hand, among either the M-II or M-III individuals, less than 25g in weight, were only the above-mentioned one (22. Og) and another M-II one (24.3 g, testicular value of 44.0), out of all males (N=119) obtained from December 1975 to November 1978. The two specimens were captured in February and April of 1976, respectively.

Judging from these facts, males weighing 25g and over were regarded as adults in this study; and also all the M-I individuals do not participate in reproduction, the M-II ones are composed of the participants and non-participants, and almost all the M-III ones seem to be fecund.

2. Breeding condition of the females

 Correspondence between types of the breeding condition by autopsy and the histological observations

The females with the undeveloped uterine horns which are semi-translucent and thread-like, and with the ovaries where the corpora lutea are not visible to the naked eye (Fig. 5A) were regarded as the F I (the first type of the female). In this type, the secondary follicles were only found in the ovaries (Fig. 5B), and the endometrium and myometrium were very thin, and the uterine lumen formed merely a simple and narrow duct (Fig. 5C); the vulva was not perforated and the teats were inconspicuous.

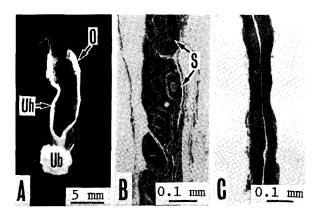


Fig. 5. Photographs showing the reproductive organ of a F I individual. A, ventral view of the undeveloped organ in its whole appearance; B, median section of the right ovary; C, longitudinal section of the right uterine horne. 0, ovary; S, secondary follicle; Ub, urinary bladder; Uh, uterine horn.

The females with the developed but depressed uterine horns and lacking both the placental scars and the corpora lutea in appearance (Fig. 6A) were decided to belong to the F-II. In this type, the tertiary follicles existed in the ovaries (Fig. GB), and the endometrium and myometrium became thick (Fig. 6C); the vulva was closed, but the teats were fairly visible.

The females with the thick swollen uterine horns and the visible corpora lutea, or with uterine swellings including embryos in addition to the corpora

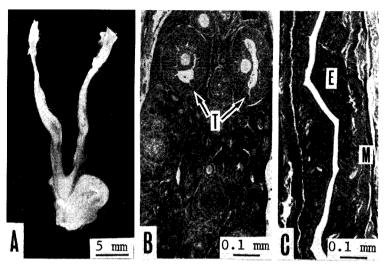


Fig. 6. Photographs showing the reproductive organ of a F-II individual. A, ventral view of the developed organ, including the depressed uterus, in its appearance; B, median section of the right ovary; C, longitudinal section of the right uterine horn. E, endometrium; M, myometrium; T, tertiary follicle.

lutea, or with placental scars in the uterine horns but lacking the corpora lutea were defined as the F-III.

The F-III individuals with the visible corpora lutea were divided roughly into the three following stages by histological changes. First, in the females at the early development stage, the presence of embryos could not be visually

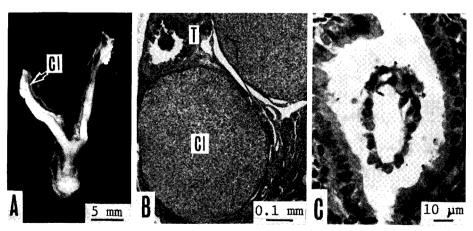


Fig. 7. Photographs showing the reproductive organ of a F-III individual. A, ventral view of the well-developed organ, including the thick and swollen uterus and the ovary with corpora lutea, in its whole appearance; B, longitudinal section of the right ovary; C, section of a free blastocyst in the uterine lumen. Cl, corpus luteum; T, tertiary follicle.

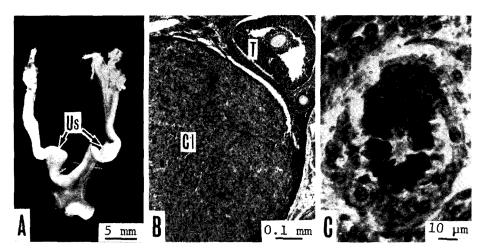


Fig. 8. Photographs showing the reproductive organ of a F-III individual. A, ventral view of the organ, including the uterus with swellings due to the presence of embryos and the ovary with corpora lutea, in its whole appearance; B, cross section of the right ovary; C, section of an egg cylinder immediately after implantation. Cl, corpus luteum; T, tertiary follicle; Us, uterine swelling.

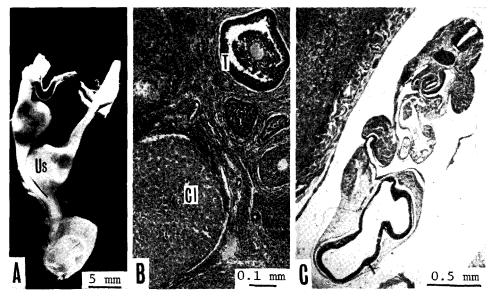


Fig. 9. Photographs showing the reproductive organ of a F-III individual. A, ventral view of the organ, including the uterus with large swellings and the ovary with the corpora lutea, in its whole appearance; B, longitudinal section of the right ovary; C, approximately horizontal section of an about 25-somite embryo. Cl, corpus luteum; T, tertiary follicle; Us, uterine swelling.

determined (Fig. 7A); the developed corpora lutea, however, were observed in the ovaries (Fig. 7B), and the endometrium became moderately enlarged and free blastocysts existed in the uterine lumen with many mucosal folds (Fig. 7C); in almost all the females at this stage, the vulva was not perforated, and the presence or absence of placental scars and the conspicuousness of the teats varied with the individuals. Secondly, in the females at the early implantation stage, pregnancy was presumed in appearance from small but macroscopically visible swellings of the urerus (Fig. 8A); the well-developed corpora lutea and tertiary follicles were observed in the ovaries (Fig. 8B), and ova at the egg cylinder stage immediately after implantation were found in the uterus (Fig. 8C); the vulva still remained closed. Lastly, for the females with more developed embryos, large uterine swellings not only were seen in autopsy but also were palpated to some extent even through the pelage (Fig. 9A); the developed corpora lutea (Fig. 9B) and the about 25-somite embryo with the differentiated organs (2.3 mm in the overall length) (Fig. 9C) were recognized: the vulva of almost all the females was closed.

In addition, the situation of the uterine horns and ovaries in the females with the placental scars but lacking the corpora lutea, was similar to that of the F-II. Among the F-III individuals, there were also one lactating female with mature follicles, two with a vaginal plug and an old individual having the atrophied ovaries despite vaginal perforation.

2) Relationship between the breeding condition and the body weight

A minimum weight of the pregnant females was 19.2 g including two egg cylinders whose weight is negligible. This female had inconspicuous teats and unperforated vulva. Only three females less than 25g (containing embryos) belonged to the F-III, including the above individual, which were captured in April and June of 1976. Also, the females less than 25 g occupied 72.2 % of the F-I individuals (N=22) obtained from December 1975 to November 1976. Thus, the females weighing 25g and over were regarded as adults as in the males; and also all the F-I and F-II voles have not participated in reproduction and only the F-III voles have been reproductively active.

3. Breeding season

The breeding season at Mt. Hitome became clear on the basis of the above results. The number of the individuals belonging to each type by the sex is shown in Table 2 for the voles weighing 25g and over and obtained from December 1975 to November 1978. In the males, the M-III individuals were collected in December 1975, from February to June, September and October of 1976, April, June and December of 1977, and February and October of 1978. In the females, no macroscopically pregnant one was captured from December 1976 to December 1977. All months when the F-III individuals appeared, however, coincided with those when the M-III ones did. The occurrence pattern of the F-I and F-II ones also resembled that of the M-I and M-II ones, respectively. Consequently, the vole's breeding mainly occurred in spring and fall.

Table 2. The number (occurrence frequency %) of individuals belonging to each type of the breeding condition in adult *Microtus montebelli* collected from December 1975 to November 1978.

3.6 .1	Male					Female		
Month	M-I	M-II	M-III	Total	F-I	F-II	F-III	Total
1975								
Dec.	3 (33.3)	5 (55.6)	(11'1,	9	6 (50, 0)	4 (33.6)	2 (16.7)	12
1976		•						_
Feb.	(0)	2 (66. 7)	1 (33. 3)	3	0 (0)	1 (50.0)	1 (50.0)	2
Mar.	0 (0)	1 (50.0)	1 (50.0)	2	0 (0)	1 (50.0)	1 (50.0)	2
Apr.	0 (0)	(0)	11 (100)	11	(0)	(20.0)	(80.0)	5
May	1 (33.3)	1 (33. 3)	(33.13)	3	(25fO)	(5020)	1 (25. 0)	4
June	3 (17. 6)	9 (52. 9)	5 (29. 4)	17	0 (0)	5 (25. 0)	15 (75. 0)	20
Aug.	(20, 0)	(80. 0)	(0)	5	(33.3)	2 (66.7)	(0)	3
Sep.	(0)	(16.17)	(8353)	6	(0)	(3323)	4 (66.7)	6
Oct.	0 (0)	(50.10)	(50.10)	2	0 (0)	(50.10)	(50.10)	2
Nov.	1 (50.0)	(50.10)	0 (0)	2	1 (100)	0 (0)	0 (0)	1
Dec.	(50, 0)	(50.10)	0 (0)	2	0 (0)	(1002)	0 (0)	2
1977	2			4	0		0	3
Apr.	(0)	(25.0)	(7530)	4	0 (0)	(33.13)	(66.7)	
June	0 (0)	(50 . 0)	(5010)	2	(33.13)	(33.13)	(33.13)	3
July	(2222)	(7778)	(0)	9	0 (0)	(100)	(0)	2
Aug.	(50.10)	1 (50.0)	0 (0)	2	(50.10)	(5010)	0 (0)	2
Oct.	0 (0)	(100)	0 (0)	1	0 (0)	1 (100)	(0)	1
Dec.	0 (0)	(50.0)	(50.10)	2	0 (0)	1 (50.0)	1 (50.0)	2
1978	ā			2	0	4	,	5
Feb.	0 (0)	(33.13)	(6627)	3	0 (0)	(20.0)	(80, 0)	
Apr.	1 (50.0)	(50.10)	0 (0)	2	0 (0)	(100)	0 (0)	2
June	3 (100)	0 (0)	0 (0)	3	1 (50.0)	(50.10)	(0)	2
Aug.	0 (0)	3 (100)	0 (0)	3	1 (50.0)	1 (50. 0)	0 (0)	2
Oct.	(0)	(0)	(100)	2	0 (0)	0 (0)	(100)	1
Nov.	0 (0)	(100)	0 (0)	1	(0)	(0)	(0)	0

DISCUSSION

1. Criteria of the breeding condition

The presence of sperm in the caudal epididymis is frequently taken as indicative of fecundity in males of Microtus species (Jameson, 1950). The M-III individuals with testicular and/or epididymal spermatozoa (Table 1), therefore, probably participate in reproduction, and it is possible to guess the breeding season from the occurrence of such M-III individuals. It also seems likely that the M-II ones with sperm are at the beginning or at the end of the breeding season because of the presence of a few sperm. The fact that the testis size is not always related to the presence or absence of sperm (Fig. 2) appears to be caused by reason that the time of spermatogenesis varies to some extent with the individuals and/or the seasons. In this connection, Kanamori and Tanaka (1968) suggested that the male breeding condition of the vole can be classified into four types by their reproductive activity index (the testicular value divided by the body weight). males used in this study are sorted by their method (Table 3), the relationship between the type and the presence or absence of sperm becomes more complicated and less clear than by our method (cf. Table 1). Such confusion is resulted from the low correlation between the body weight and the testicular value in the vole as shown in Fig. 4, and also from the seasonal changes in the testis size (Miyao et al., 1966; Kimura et al., 1980).

Table 3. Male breeding condition estimated by method of Kanamori and Tanaka (1968) using the same samples as in Table 1.

Sperm	Туре				
	I	II	IIJ	IV	
Presence Absence	2 12	2 22	8 2	20 1	

Due to the indistinct border of the scrotal bulge and hence a large error in its measurement, the correlation between the scrotum size and the testis length seems to become unsignificant. However, since the males having a visible scrotal bulge always belong to the M-III, it is regarded as a single valid criterion for knowing the reproductive activity of the live males in the field.

For the autopsied females, it is possible to know the actual state of their breeding condition by the appearance of the uterus and by the presence or absence of corpora lutea with the naked eye. The process of uterine repair including the remaining duration of visible placental scars after parturition, however, remains to be proved in future. On the other hand, the reproductive activity of the live females cannot be judged by the conspicuousness of the teats, because the females which had experienced parturition seem to persist their conspicuousness even in non-reproductive condition. The teats of the lactating females, however, can be distinguished from those of non-

lactating one. The vulva is perforated, of course, at the time of coitus and parturition, but the duration of vaginal opening remains unknown. Also, the at least 25-somite embryo (Fig. 9C) corresponding to the mouse embryo of ca. 10 days (Theiler, 1972) could be palpated. Taking the above fact into consideration, it is revealed that the teats which are sucked by young, perforated vulva and pregnancy detected by palpation are utilized as useful criteria, if not complete, in order to know the reproductive activity of the live females.

2. Reproductive activity in the unusually growing population

The adult body weight of voles of both sexes was 25g and over at Mt. Hitome, and this value approximately resembled the values of above 28g for the males and over 25g for the females at Kybto City (Kaneko, 1976), and above 25 g for the females at Fukushima City (Kimura et al., 1980). According to Kanamori and Tanaka (1968), however, the adult weight was 20g and over for both sexes at Sugadaira-plateau of Nagano Prefecture, being a little lighter than ours, for they adopted the minimum weight of the individuals with reproductive activity. Both the males and the females of about 20g in weight, which participated in reproduction, were obtained also at Mt. Hitome only from February to June in 1976 corresponding to the unusual growing period of the vole's population (Arai and Shiraishi, 1982). In addition, in the growth phase the individuals which are considerably heavier than in the declining phase were frequently captured (unpublished). Judging from these results, it seems likely that during the unusual growing period both sexes reached sexual maturity and participated in reproduction at the younger age than in normal years.

On the other hand, the breeding season of the vole known at present is in either mainly summer (Miyao et al., 1966), or both spring and fall (Shiraishi, 1967; Abe, 1974; Kaneko, 1976). Although the breeding season at Mt. Hitome is fundamentally spring' and fall, it varies a little with the years. For example, a long lasting reproductive activity occurred during the period of December 1975 to June 1976 including the winter (Table 2); since some individuals belonging to the M-III and F-III were caught on 20 October and 25 November in 1975, it had in fact begun at latest in the fall of 1975. We, therefore, believe that such a long breeding season and the participation of the younger voles in reproduction played important roles in the vole's outbreak in June 1976 (Arai and Shiraishi, 1982). Also, the breeding during the winter only in 1977-78 seems to be attributed to both the delay in onset of the fall breeding in 1977 and the early start of the spring breeding in 1978.

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