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Intensity of male reproduction in Brandt's vole *Microtus brandti*

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A new method based on polygonal regression analysis to investigate the relationship between the testis (or seminal vesicle) length and body mass were used to study the dynamics of male reproductive intensity of Brandt's voles *Microtus brandti* (Radde, 1861). The results showed that the turning (join) points which could be regarded as the minimum body masses commencing sexual development increased from spring to autumn. The slope rates of regression equations which represent the sexual growth rate decreased from spring to autumn. This indicated that the sexual development of males had obvious seasonality similiar to that of the females. By comparing two years, we could find that the slopes in 1987 (population increasing year) were significiantly higher than those of 1988 (population decreasing year). The slopes and the percentage of the voles with body mass larger than the turning point might be reliable indexes of male reproductive intensity for studies of population dynamics in rodents.

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Introduction

Brandt's vole *Microtus brandti* (Radde, 1861) is the major pest rodent in the pasture of typical steppe in Inner Mongolia. The population ecology and control of this rodent pest has been intensively studied in the past 40 years, such as physiological age structure, frequency of producing litters, growth rate of cohorts, age at sexual maturation, mean litter size (Anti-epidemic and Health Station 1975, Zhang and Zhong 1979, Liu 1992, Zhou *et al.* 1992, Liu and Sun 1993a, b). Those results were mostly based on females. Yet very little is known about the male reproduction ecology and the role of males in the population reproduction. Only Zhang and Zhong (1979) studied the monthly changes of percentage of the voles with enlarged seminal vesicles or scrotal testes in the population. We used the polygonal regression to investigate the relationship between sexual organ growth and body mass. Two male reproductive intensity indexes were presented here.

Material and methods

The filed site was located at Hongguang Muchang (42°18'N, 115°00'E, altitude 1346.3 m a.s.l.), Zhengxiang Baiqi, Xilinggulei League, Inner Mongolia Autonomous Region. This site belongs to the zone of arid steppe with needle grasses and peashrubs. We obtained more than 100 voles per half month by steel snap traps from April to October 1987 and from March to September 1988. The snap traps (100 × 45 mm) without any bait were placed on all burrow openings of the sample area at day time. Each trapping period lasted two days in summer or three days in spring and autumn. Captured voles were collected every two hours and carried to a field laboratory to be necropsied directly. The following data were collected: body mass to the nearest 0.5 g, body length to the nearest 0.5 mm, testis and seminal vesicle length to the nearest 0.1 mm, net body mass (body mass without abdominal viscera) to the nearest 0.5 g.

According to the distribution of the net body masses, the voles were classified as overwintered and born in this year (Liu and Sun 1993a). Because too few voles born in this year were captured in late half of May 1988 ($n = 7$), they were combined to early June (called May–June).

The rodents grow and develop continuously, but at an uneven rate. The sexual organs of young animals are undeveloped, although body mass increases rapidly. After sexual maturation is attained, the sexual organs grow slowly or remain at a relatively constant state. The sexual organs (testis or seminal vesicle) were divided into three groups: "Mature" – largest testis; "Rudimental" – lightest body mass, the testes remained thin and small and increased slowly, relative to the increase in body masses that was rapid; "Growth" – the intermediate group, the testis increased rapidly. Our problem was how to classify these three groups by using a mathematical method, and to determine the turning point between the groups.

The mean length of the testis of overwintered voles could be regarded as the turning point between "Growth" and "Mature" because the overwintered voles were all sexually mature (see Table 1). In the present study, "Mature" (excluding overwintered voles) is neglected because some sample sizes are too small. The numbers of deleted voles whose testes were longer than the mean of overwintered voles were as follows: 0 (late May and early June), 2 (June–July), 8 (July–Aug), 10 (Aug–Sep) in 1987 and 0 (May–June), 2 (June–July), 12 (July–Aug), 2 (Aug–Sep) in 1988.

The linear regression could be used to analyze "Growth" and "Rudimental" states with respect to body mass. But the problem remaining to be solved is how to determine the turning point between two groups. For a data set of size m (X_i, Y_i) $(i = 1, 2, \ldots\ldots, m)$ characterized the two random variables Y (= testis or seminal vesicle length) and X (= body mass), where the data are ordered such as $X_1 < X_2 < \ldots < X_m$, we could select one turning point J, such that $X_1 < J < X_m$. Let

$$Y = \begin{cases} A_1 + K_1X & \text{for } X \le J \\ A_2 + K_2X & \text{for } X > J, \end{cases} \tag{1}$$

where: $A_1 + K_1T = A_2 + K_2T$. K_1 and K_2 stand for the slopes of the linear regressions. The polygonal regression equation with one turning point J could be expressed as:

$$H_{(X)} = C_0 + C_1X + C_2\,(X - J)_+ \tag{2}$$

where: $(X - J)_+ = \begin{cases} 0 & \text{for } X \le J \\ X - J & \text{for } X > J, \end{cases}$

C_0, C_1 and C_2 are parameters remaining to be determined. If we let $Z_1 = X$, $Z_2 = (X - J)_+$, then Eq. (2) may be expressed as:

$$H_{(X)} = C_0 + C_1Z_1 + C_2Z_2 \tag{3}$$

So the polygonal regression Eq. (2) could be transformed to multivariate linear regression Eq. (3).

Then turning points were determined by using the method of exhaustion. That is to search for the integral number point (J_0) such that $X_i < J_0 < X_m$, minimizes the residual sum of squares $\Sigma\,(Y_i - C_0 - C_1Z_{i1} - C_2Z_{i2})^2$ (or maximize the F value in the significance test). Where Z_{i1} and Z_{i2} were converted from the original data by the formula $Z_1 = X$, $Z_2 = (X - J)_+$. In other words, we regard all the integral numbers which are larger than X_i and smaller than X_m as the turning point and calculate multivarite regress equations, the residual sum of squares and F values. By comparing all F values or values of the residual sum of squares, the turning point could be selected.

The turning point could be regarded as the minimum body mass in which sexual organs of the young began to develop rapidly. The slope of regression equation was regarded as the growth rate of sexual organs. So there is a turning point (in biology not in mathematics) only when $K_2 > K_1$. If the turning point (biological) is not existing, the linear regression could be used instead of polygonal regression and expressed as

$$Y_3 = A_3 + K_3X \quad (4).$$

Fang and Sun (1989) investigated the population dynamics of Brandt's voles at the same study site and indicated that population was increasing in 1987 and decreasing in 1988.

Results

The body masses, testis and seminal vesicle length of overwintered voles and voles born of this year were listed in Table 1 and 2, respectively. All captured overwinterd voles had enlarged seminal vesicles and scrotal testes. From April to July in two years, there were no significant differences between the mean length of testes (seminal vesicles) for overwintered animals (e.g. April to July, testis: in 1987, $t = 0.1116$, $p > 0.01$; in 1988, $t = 1.9236$, $p > 0.01$ respectively; seminal vesicle: in 1987, $t = 1.9688$, $p > 0.01$; in 1988, $t = 1.7934$, $p > 0.01$). So we could conclude that the length of sexual organs of overwintered voles changed very little through the breeding season.

The polygonal regression equations reached a significant level ($p < 0.001$, Tables 3 and 4). There were significant differences between K_1 and K_2 of equations in Tables 3 and 4 ($p < 0.001$). This indicated that the turning points were existing in those months. But in early June of 1987, K_1 (0.9772) were larger than K_2 (0.2877) (J = 10) and there was no significant differences between K_1 (0.1105) and K_2 (= 0.3207) in late May (J = 9). So, the (biological) turning points were not

Table 1. The range of body mass, mean ± SE length of testis and seminal vesicle length in overwintered voles *Microtus brandti*.

Year	Month	Sample size (*n*)	Body mass (g)	Mean length of testis (mm)	Mean length of seminal vesicle (mm)
1987	April	26	34.5–75.0	14.79 ± 1.21	16.92 ± 2.18
	May	92	35.5–78.0	14.92 ± 1.37	17.22 ± 2.52
	June	23	47.5–67.5	15.33 ± 1.07	17.72 ± 2.28
	July	19	48.0–68.0	14.90 ± 0.79	18.26 ± 2.31
1988	April	84	29.0–69.0	14.69 ± 1.38	17.29 ± 3.79
	May	101	40.0–71.0	15.04 ± 1.09	18.03 ± 2.84
	June	46	42.0–73.0	15.14 ± 1.14	18.91 ± 2.60
	July	16	49.0–58.0	15.34 ± 1.21	19.05 ± 3.56

existing in late May and early June. The reason was that there were too few voles with body mass smaller than the (mathematics) turning point (late May, $n = 3$). So, the linear regression equations were present in Table 3 and also the lightest body mass was considered as the turning point. This indicated that all the young appearing on the ground had commenced sexual growth. The turning points increased from spring to autumn significantly (Tables 3 and 4, $p < 0.001$; mean square successive difference test, Neumann 1941, Seber 1977).

Table 2. The range of body mass, testis length, and seminal vesicle length of Brandt's voles born in this year (excluding "Mature").

Year	Month	Body mass (g)	Seminal vesicle length (mm)	Testis length (mm)
1987	Late May	7.0–25.0	2.0– 6.0	3.0–10.5
	Early June	8.0–37.0	1.0–10.0	3.5–14.0
	June–July	5.0–39.0	1.0–13.5	3.5–10.5
	July–Aug	5.5–39.0	1.5– 8.5	1.5– 9.5
	Aug–Sep	11.0–42.0	1.5– 7.5	2.5– 8.0
1988	May–June	7.0–28.5	1.0–11.0	2.0–11.5
	June–July	8.0–42.0	1.0–14.0	2.0–13.5
	July–Aug	10.0–39.0	1.5– 8.5	1.5– 8.5
	Aug–Sep	11.0–35.0	2.5– 8.0	2.0– 6.5

Table 3. Polygonal regression equations of testis length (Y) and body mass (X) of Brandt's voles (Y_3 present linear regression equation).

Year	Month	Regression equations	*n*	*F* value	*p*	Minimum body mass at sexual maturation
1987	Late May	$Y_3 = 1.9715 + 0.3166X$	85	197.46	< 0.001	15.88
	Early June	$Y_3 = 2.3634 + 0.2964X$	78	120.46	< 0.001	15.64
	June–July	$Y = 2.5661 + 0.0720X$, $X \le 23$				
		$Y = -0.8126 + 0.2189X$, $X > 23$	162	51.91	< 0.001	37.40
	July–Aug	$Y = 3.6575 + 0.0327X$, $X \le 29$				
		$Y = -1.8647 + 0.2231X$, $X > 29$	221	64.04	< 0.001	39.73
	Aug–Sep	$Y = 3.3536 + 0.0321X$, $X \le 31$				
		$Y = -0.9027 + 0.1694X$, $X > 31$	250	82.07	< 0.001	46.65
1988	May–June	$Y = 4.1441 + 0.0972X$, $X \le 13$				
		$Y = 1.3864 + 0.3086X$, $X > 13$	45	110.96		18.15
	June–July	$Y = 2.7005 + 0.1429X$, $X \le 20$				
		$Y = 1.0525 + 0.2253X$, $X > 20$	91	28.44	< 0.001	26.40
	July–Aug	$Y = 4.6785 + 0.0387X$, $X \le 24$				
		$Y = 0.4420 + 0.2152X$, $X > 24$	145	31.48	< 0.001	30.47
	Aug–Sep	$Y = 4.7936 + 0.0403X$, $X \le 28$				
		$Y = -1.2441 + 0.1668X$, $X > 28$	137	8.02	< 0.001	49.43

K_1 of all the polygonal regression equations was much smaller. Tables 3 and 4 also indicate that K_2 had seasonal variations significantly (ANOVA, Table 3: 1987, $F = -111.67$, $p < 0.001$; 1988, $F = -31.10$, $p < 0.001$; Table 4: 1987, $F = -55.47$, $p < 0.001$; 1988, $F = -6.93$, $p < 0.05$). There were significant differences

Table 4. Polygonal regression equations of seminal vesicle length (Y) and body mass (X) of Brandt's voles.

Year	Month	Regression equations	*n*	*F* value	*p*
1987	Late May	$Y = 1.7313 + 0.0787X$, $X \leq 10$			
		$Y = 0.5979 + 0.1921X$, $X < 10$	84	75.67	< 0.001
	Early June	$Y = 2.1115 + 0.0922X$, $X \leq 24$			
		$Y = -5.8522 + 0.4241X$, $X > 24$	78	94.44	< 0.001
	June–July	$Y = 2.5914 + 0.0271X$, $X \leq 26$			
		$Y = -1.8842 + 0.1993X$, $X > 26$	162	37.23	< 0.001
	July–Aug	$Y = 1.5654 + 0.0503X$, $X \leq 32$			
		$Y = -2.7194 + 0.1842X$, $X > 32$	221	113.38	< 0.001
	Aug–Sep	$Y = 1.4367 + 0.0303X$, $X \leq 31$			
		$Y = 0.8220 + 0.1061X$, $X > 31$	250	15.82	< 0.001
1988	May–June	$Y = 2.3823 + 0.0725X$, $X \leq 23$			
		$Y = -2.6581 + 0.2916X$, $X > 23$	45	8.02	< 0.01
	June–July	$Y = 1.2553 + 0.0818X$, $X \leq 32$			
		$Y = -2.7514 + 0.2070X$, $X > 32$	91	8.66	< 0.001
	July–Aug	$Y = 1.9854 + 0.0795X$, $X \leq 27$			
		$Y = 1.6320 + 0.0926X$, $X > 27$	145	9.94	< 0.001
	Aug–Sep	$Y = 0.1989 + 0.0020X$, $X \leq 30$			
		$Y = -0.5377 + 0.0932X$, $X > 30$	137	2.09	< 0.02

Table 5. The percentage of Brandt's voles with body mass larger than the turning points (A) and with testis longer than 7 mm (B) in the population.

Month	1987				1988			
	A		B		A		B	
	% of all voles	% of voles born in this yr	% of all voles	% of voles born in this yr	% of all voles	% of voles born in this yr	% of all voles	% of voles born in this yr
Late May	81.1	97.6	19.0	22.4				
Early June	76.8	97.4	62.6	79.5	25.8	66.7	20.7	53.3
June–July	55.2	61.6	26.2	29.3	34.5	44.1	30.3	38.7
July–Aug	28.5	28.8	7.8	7.9	20.6	21.0	18.8	19.1
Aug–Sep	33.9	33.9	8.4	8.4	15.3	15.3	13.1	13.1

in the slopes (K_2) between 1987 and 1988 (ANOVA, Table 3; $p < 0.001$; Table 4; $p < 0.001$). The sexual organs of voles in 1987 grew and matured fast and early. This trend also has been shown by the females (Liu and Sun 1993b).

Males were considered to be sexually mature (could produce mature sperms) if their testes were longer than 7 mm (Zhang and Zhong 1979, Zhang 1989). Then using this value (Y_2 or $Y_3 = 7$) to solve the equations, we could obtain the minimum body masses of male voles at sexual maturation (Table 3). The percentage of the voles with body mass larger than the turning point and that of the voles with testis longer than 7 mm also could be calculated (Table 5). The minimum body mass of male voles at sexual maturation and the percentages of Brandt's voles with body mass larger than the turning points and with testes longer than 7 mm in the population varies seasonally (Table 5). In females, the pregnancy rates also show the obvious seasonality (Table 5) (Liu and Sun 1993b). Liu and Sun (1993b) also indicated that the voles began breeding earlier in 1987 and the pregnancy rates of 1987 were relatively higher than those of 1988. The percentages of reproductive males also showed the same trend. Hence, reproductive activity of the two sexes is consistent.

Discussion

In studies of rodent ecology, the reproductive conditions of females has been emphasised and widely investigated. The reproductive intensity of male rodents has been rather neglected or overlooked (Sun *et al.* 1977, Keller 1985). The main reasons might be that there are no reliable and quantitative indexes of male rodents reproductive activity. On the other hand, the female reproductive profermance is probably most important demographically. This had brought some authors' attentions (Kalera 1957, Sun *et al.* 1977, Xia *et al.* 1982, Zhang 1986, 1987, 1989).

The monthly variations of mean weight (length) of testes or seminal vesicle were used as indicators of the male reproduction in many studies (Zhang 1987, Kenagy and Barnes 1988, Hilton 1992, Mills *et al.* 1992, Wang *et al.* 1992, Zhou *et al.* 1992). Although seminal vesicles of adults could wither to varying degrees in the non-breeding season, the length of testis (or seminal vesicle) changes very little during the breeding season (Sun *et al.* 1977, see also Table 1). Some published papers had also used the percentage of the rodents with enlarged seminal vesicles or scortal testes in the population to represent male breeding intensity (Zhang and Zhong 1979, Beacham 1980, Xia *et al.* 1982, Mills *et al.* 1992, Wang *et al.* 1992, Zhou *et al.* 1992). As long as we accept scortal testes or enlarged seminal vesicles as the standards for grouping non-reproductive and reproductive rodents, variation of these two percentages only indicate the changes of the population structure.

The scattered plots of the testes weight against body mass without viscera had been applied to show the tendency of the male breeding intensity (e.g. Zhang 1987, 1989), but the data were not analyzed statistically. Zhang (1986) used linear regression to investigate the relationship between testis weight and body mass without viscera in striped hamsters, but he did not turn sufficient attention to the scarcity of his data. His result could not show the pattern of male reproduction as revealed in the present paper.

Another approach was taken by Sun *et al.* (1977) who grouped *Clethrionomys rufocanus* and *Apodemus* sp. samples into weight classes of 3 g and divided the testis (seminal vesicle) into three groups. Then the intermediate group was analyzed by using the linear regression whereas the other two groups were not analyzed. But this determination of turning points was done objectively, hence the turning point was not trustworthy.

Liu and Sun (1993a, b) indicated that Brandt's vole had a short lifespan (about one year) and the population had very high turnover rates. Before the latter half of May, the population was composed only of overwintereds. As soon as the voles born in this year entered the population, they replaced the overwintereds rapidly. Four cohorts were produced from the latter half of April to the first half of September. The voles born in spring grew and matured very rapidly and reached sexual maturation to begin breeding at 3 or 4 weeks of age. These early maturing animals had almost disappeared from the population in autumn. Whereas the young born in the middle or late summer and autumn grew slowly and did not reach sexual maturity in fall and resumed growth again the following spring. These overwintereds (having more than 30 weeks of age) constitute the breeding population in early April.

Our results also indicated that the growth and sexual development of males have an obvious seasonality similar to that of the females. This seasonality could be indicated by three aspects. The first was the turning points. In spring the voles with a body mass of 9 g initiated sexual development and in autumn the voles only with body mass larger than 31 g could begin sexual growth (Table 3). The second was the growth rate of sexual organs (K_2). The growth rate could reach 0.32 in spring and in contrast only 0.17 in fall (Table 3). The third was the minimum body mass at sexual maturation. In late May of 1987, the voles with a body mass of 15.9 g were able to produce mature sperm. But in July–August of 1987, this minimum body mass reached 39.7 g (Table 3).

This seasonality also indicated that the vole's physiological age differed from the chronological age. As the voles having the same body mass in different seasons may belong to different age group. So one should be most cautious to use the chronological age standards such as body mass, body length, net body mass, morphology of skull and tooth to categorize the Brandt's voles into four groups such as juvenile, subadult, adult one and two. Zhang and Zhong (1979) indicated that 35% of the juvenile group in May, 1975 were pregnant. This shows that it is necessary to determine the physiological age group instead of the chronological age group or at least to use different body mass (length) standards in different seasons (Liu and Sun 1993a).

In conclusion, male voles have the same seasonal reproduction pattern as the females. Our analysis also has demonstrated that the growth rates of male sexual organs and the percentage of voles with testes longer than 7 mm or body masses larger than a given turning point could be used as indexes of male reproductive intensity.

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