Ann. Zool. Fennici 22:303-308. 1985

Sexual maturation in *Clethrionomys*

Torgny O. Gustafsson

Gustafsson, T. 1985: Sexual maturation in *Clethrionomys*. — Ann. Zool. Fennici 22:303-308.

Laboratory work concerning two aspects of sexual maturation, social regulation of maturation in males and fertility during the pubertal period in females, is summarized.

Social regulation of puberty of male *Clethrionomys glareolus* was studied in cyclic animals from North Sweden and non-cyclic ones from South Sweden. Presence of other males influence male puberty differently in these animals. If young males are kept in groups of two or four, puberty is delayed in cyclic, but not in non-cyclic males. If, on the other hand, young males are exposed to unfamiliar mature males, non-cyclic males are suppressed, while cyclic ones are unaffected, or possibly even stimulated. These results indicate that different mechanisms for density regulation of maturation have evolved in these two populations.

Pubertal female *Clethrionomys* often show a period of sterility with several non-fertile matings before the first fertile one. It is not clear whether the phenomenon is density-dependent. Field data for *C. glareolus* indicate that a period of sterility is common in both non-cyclic and cyclic populations, and that it is equally common in all years in cyclic populations. Studies on other species suggest that the phenomenon is more common in peak years.

Laboratory studies show that this probably is not due to pregnancy block. Sterile matings are seen also when the studied male is left with the female after mating. Young females appear to need more copulatory stimulation than one normal mating for activation of the corpora lutea of pregnancy. The sterile matings may be a method of accelerating puberty: the fertility of young females is higher if they have experienced one or two sterile matings.

Torgny O. Gustafsson, Department of Biology, Division of Zoology, University of Oslo, P.O.Box 1050, Blindern, N-0316 Oslo 3, Norway. Present address: Jättegatan 21, S-284 00 Perstorp, Sweden.

1. Introduction

Both the magnitude of population fluctuations and the maximum reached density vary greatly among *Clethrionomys* populations. In accordance with this, the social regulation of maturation of young of the year differs much among populations. An almost total inhibition of sexual maturation of young of the year is seen in almost all dense populations. This is true both for cyclic populations of *C. rufocanus* (Kalela 1957) and of *C. glareolus* (Gustafsson 1983) and for the *C. rutilus* population studied by Koshkina & Korotkov (1975) where the population density is exceptionally high in most years, but fluctuations in density are small and irregular. The index

$$s = \sqrt{\operatorname{var}(\log(N))}$$

where N is the density in corresponding seasons in successive years (Williamson 1972, Stenseth 1977) measures the magnitude of population fluctuations. In the above mentioned population of *C. rutilus*, the index is 0.29 to 0.32 dependent on habitat, when calculated for spring densities. During the course of the breeding season, the index decreases to only 0.07 in closed taiga and 0.18-0.22 in the other habitats. This stabilization of autumn density is probably mainly due to regulation of sexual maturation. In the strongly cyclic North Swedish *C. glareolus* population, there is no evidence of such stabilization in spite of a density-dependent suppression of maturation; instead, the spring index is smaller (0.77) than the autumn index (0.86).

The *C. glareolus* population on Crab Apple Island in Poland (Bujalska 1975) has some similarities with the Soviet *C. rutilus* one. The density is high in all years, numbers were remarkably constant, and removal of part of the spring born cohort resulted in a larger part of the subsequent cohorts maturating, and in a compensation of total number in autumn. The main difference with respect to maturation is that in this population most of the early-born cohort reached maturity in all years, and that a regulation of maturation was only seen in later cohorts.

In populations which never reach high densities, and at lower density in other populations, most or all young born in early summer maturate rapidly, and if there is a density-dependent regulation, this is only observed for animals born during mid-summer (Fuller 1979). Several populations never reach densities sufficiently high to inhibit sexual maturation. For example, most populations of *C. glareolus* in western Europe (Great Britain, Denmark, South Sweden; see Gustafsson 1983, Jensen 1982) do not reach sufficiently high densities.

2. Evolution of density-dependent regulation of sexual maturation

The pattern emerging from the survey summarized in the previous section may have consequences for the evolution of density-dependent regulation of maturation. It seems plausible that density-dependent regulation is more likely to evolve in high density populations and in cyclic populations which reach high peaks than in low density populations. A series of laboratory experiments on *C. glareolus* indicate that such differential evolution has, in fact, taken place, and that there exist quantitative as well as qualitative differences in the social regulation of maturation between a cyclic population and one which is stable at a fairly low density.

In these experiments, cyclic animals from North Sweden and non-cyclic animals from South Sweden (localities are described in Gustafsson (1983)) were used. Experiment I and II were performed at the University of Lund (Sweden), with a non-cyclic colony established in 1974 and two cyclic ones, established in 1976 and 1977. Experiment III was performed at the University of Oslo (Norway) with new non-cyclic (founders caught in 1981) and cyclic (founders caught in 1982) colonies from the same localities. Results from the third experiment are reported for the first time in this paper. Reproductive performances in the colonies kept at University of Lund are described by Gustafsson et al. (1983b). In all experiments, animals were kept in a laboratory environment, with a long day (18L:6D) and standard laboratory mouse food.

Table 1. Body weight and weights of reproductive organs and adrenals in 60 d old male bank voles from South and North Swedish laboratory colonies as affected by group size. Weights are given as mean ± S.E. Group I = one male per cage, group II = two males per cage and group III = four males per cage.

Origin of animals	Group	*n*	Body wt (g)	Testes wt (mg)	Seminal vesicle wt (mg)	Adrenal wt (mg)
South Sweden	I	23	20.6 ± 0.4	592.4 ± 15.6^c	176.4 ± 9.8	3.8 ± 0.2
	II	24	21.1 ± 0.5	635.1 ± 13.8	179.9 ± 8.1	3.7 ± 0.1
	III	23^x	21.4 ± 0.6	589.6 ± 16.4^c	194.4 ± 7.7	3.5 ± 0.1
North Sweden 1976 colony	I	30	18.3 ± 0.5	358.2 ± 51.7	88.3 ± 17.9	4.5 ± 0.1
	II	27	17.2 ± 0.5	349.1 ± 53.6	83.2 ± 19.9	4.4 ± 0.2
	III	36	17.2 ± 0.4	260.1 ± 47.6	51.2 ± 14.0^b	4.2 ± 0.1^b
North Sweden 1977 colony	I	27	19.3 ± 0.5	421.7 ± 46.3	67.1 ± 11.6	4.3 ± 0.2^c
	II	26	18.9 ± 0.6	450.6 ± 67.0	77.6 ± 15.2	4.9 ± 0.2
	III	28	17.3 ± 0.4^{a,c}	210.1 ± 43.7^{a,c}	26.2 ± 9.4^{a,c}	4.4 ± 0.2

x One male with a testicular infection was excluded
a Different from group I, $p < 0.01$, Mann-Whitney U-test
b Different from group I, $p < 0.05$, Mann-Whitney U-test
c Different from group II, $p < 0.05$, Mann-Whitney U-test

Table 2. Results of experiment II. Effect of daily confrontations with unknown animals on 40 d old male bank voles. Each experiment consists of *N* littermate groups, with one littermate male in each treatment. Body weight and weights of reproductive organs are given as mean ± S.E.

Treatment	*N*	Body wt (g)	Testes wt (mg)	Seminal vesicle wt (mg)	Adrenal wt (mg)
with an adult male	15	19.6 ± 0.5^b	510.3 ± 28.6^b	64.3 ± 10.8^b	3.2 ± 0.1^b
with an adult male, changed daily	15	16.4 ± 0.5^b	320.6 ± 45.7^b	23.2 ± 8.1^b	3.9 ± 9.2^b
with an adult female	18	18.4 ± 0.5^b	417.2 ± 42.5^a	54.6 ± 10.8^a	3.0 ± 0.1^a
with an adult female, changed daily	18	17.4 ± 0.5^b	297.7 ± 48.5^a	30.4 ± 10.3^a	3.3 ± 0.1^a
with a juvenile male	24	19.5 ± 0.3^b	535.0 ± 11.5^b	82.2 ± 7.3^a	3.2 ± 0.1
with a juvenile male, changed daily	24	18.4 ± 0.3^b	483.2 ± 19.5^b	63.0 ± 8.5^a	3.1 ± 0.1

Only within experiment statistical comparisons were made. Means denoted with the same letter are significantly different, a: $p < 0.05$, b: $p < 0.01$, Wilcoxon's signed-ranks test.

2.1. Experiment I (Gustafsson et al. 1983a)

This experiment compares the effect of group size on sexual maturation in males from cyclic and non-cyclic populations. Young voles were kept in groups of 1, 2 and 4 from weaning (day 18) until 60 days old. All non-cyclic males in this experiment were mature when 60 days old, and no difference between groups could be seen (Table 1). In cyclic animals, mean body weight and weight of sexual organs decreased with increasing group size (Table 1). Individual development was extremely variable in the cyclic animals, and both fully mature and completely inhibited animals were seen in all treatments.

2.2. Experiment II (Gustafsson 1984)

The aim of experiment II was to study the influence of unfamiliar animals on male sexual maturation. In this experiment only non-cyclic animals were used. During maturation (from 18 to 40 days of age) young males were kept either paired with the same individual the whole period, or were paired, in their own home cages, with a new animal each day. Response to adult males, adult females and young males was studied. A control group of isolated young was included in the experiment with effect of adult males.

Daily contacts with unfamiliar animals caused a marked retardation of sexual development (Table 2). The sex and state of maturation of the unfamiliar animal seemed unimportant, suppression was caused by contact with unfamiliar adult females and both adult and immature males. No difference was seen between isolated males, and males paired with adult males.

Thus, although less responsive to suppression when reared in groups, non-cyclic males responded markedly to contact with unfamiliar animals. This suggests that dispersal may be important in determining the degree of social inhibition of maturation.

2.3. Experiment III

This experiment had two objectives: 1) to compare the sensitivity of cyclic and non-cyclic animals to stimuli associated with dispersal, and 2) to see whether physical contact between animals is necessary for suppression. In this experiment the responses of pairs of littermate from non-cyclic and cyclic males to familiar and unfamiliar mature non-cyclic males were tested. At weaning, when 18 days old, the young males were placed in experimental cages, divided by a perforated stainless steel plate into two compartments, one for the young male and one for an adult male. In the "migration" group, the adult male was changed twice daily, whereas the control animals were exposed to the same adult individual all the time. The exchange of adult males was automatized with a system of tunnels and doors which were opened by electromagnets. When 35 days old, experimental animals were killed, and reproductive organs were dissected out and weighed.

The response was markedly different in cyclic and non-cyclic animals (Table 3). In non-cyclic animals, a picture similar to that seen in experiment II, with a suppression of maturation in those exposed to unfamiliar

Table 3. Body weight and weights of organs in 35 d old male bank voles of South and North Swedish origin, reared in different social environments. Treatments: Familiar male: Reared with an adult male but separated by a perforated metal plate. Unfamiliar male: As above, but adult male changed twice daily. Weights are given as mean ± S.E.

Origin and treatment	*N*	Body wt (g)	Testes wt (mg)	Seminal vesicle wt (mg)	Preputial glands wt (mg)
South Sweden					
Familiar male	29	17.9 ± 0.5	377 ± 20.5^a	25.5 ± 3.9^a	11.9 ± 1.2^b
Unfamiliar male	29	17.4 ± 0.5	326 ± 21.1^a	20.8 ± 3.8^a	9.9 ± 1.1^b
North Sweden					
Familiar male	28	19.1 ± 0.5	209 ± 34.0	13.4 ± 3.8	5.3 ± 0.7
Unfamiliar male	28	19.7 ± 0.5	272 ± 37.7	20.1 ± 7.0	6.8 ± 1.4

a: Different, $p < 0.025$, Wilcoxon's signed-ranks test

b: Different, $p < 0.05$, Wilcoxon's signed-ranks test

males, was seen. The cyclic males on the other hand were not suppressed by contact with other males. Instead, they tended to be somewhat better developed when exposed to unfamiliar males than when kept with the same adult, although this tendency was not significant.

3. Discussion

3.1. General

These results indicate that the cues used by North Swedish cyclic and by South Swedish non-cyclic bank voles to decide whether to maturate or not may be different. North Swedish animals may need to establish an exclusive territory, or to attain some degree of social dominance. This seems to agree with Bujalska's (1973) findings for a dense Polish island population and Viitala's (pers. comm.) findings for a Finnish population of *C. glareolus*, and with Viitala's (1977), Saitoh's (1981) and Bondrup-Nielsen's (1983) findings for *C. rufocanus* and *C. gapperi*.

Our laboratory findings suggest that an exclusive territory is less important for South Swedish *C. glareolus*. However, it is not known whether this is true in nature. The population density in South Sweden is always much lower than peak densities in cyclic populations (Gustafsson 1983) or on Crab Apple Island (Bujalska 1973), and it may be that territory related suppression of maturation has not evolved, or has been lost, in this population.

It is surprising that the stimulus causing strongest suppression of South Swedish males, contact with unfamiliar animals, was ineffective or possibly even stimulatory for North Swedish males. If real, the results of experiments I and III could be interpreted in the following way: North Swedish males need to establish an exclusive territory or at least some degree of social dominance before they can maturate. If they can achieve this, they respond positively to contact with other animals. This could also be an explanation for the larger body weight seen at peak densities of these cyclic populations (Gustafsson 1983).

When extrapolating from these laboratory findings to natural populations, it is important to consider the validity of results obtained with animals bred in captivity. One problem with establishing laboratory populations is that in most cases only a relatively small proportion of the animals brought in will breed in captivity. This might result in a large difference between the genetic make up of laboratory and field populations. Gustafsson et al. (1983) and Hansson (1985) have compared characteristics of laboratory colonies of *C. glareolus* from cyclic and non-cyclic populations. The results of these comparisons are summarized in Table 4. Some inconsistencies are seen:

1) In litter size, where Gustafsson and coworkers, but not Hansson found larger litters in cyclic animals. This is, however, consistent with results from the natural populations, where the population in Ammarnäs (Gustafsson 1983), but not the one in Jämtland (Hansson & Henttonen, 1985) had larger litters than the South Swedish one.

2) In one of the North Swedish colonies studied by Gustafsson and co-workers, mortality of young was much lower than in the others. This colony was kept in another type of cage, with a nest box.

Table 4. Comparison of laboratory bred bank voles of South Swedish (non-cyclic) and North Swedish (cyclic) origin. A: Three colonies from Revinge, South Sweden, and three from Strömsund, North Sweden (Hansson, 1985). B: Three colonies from Revinge, South Sweden, and three from Ammarnäs, North Sweden (Gustafsson et al. 1983b, Gustafsson unpubl.). Legends: + consistent difference between localities; (+) difference seen in some cases, not consistent; - no difference.

Type of difference	Study A	Study B
North Swedish animals larger	+	+
Morphological differences, North Swedish animals greyer, longer fur etc.	+	+
Larger litters in North Swedish animals	-	+
Lower survival of young in North Swedish animals	+	(+)
Fewer North Swedish animals reproduce	+	+
North Swedish animals more active	+	+
Marked differences in reproduction between wild caught animals and F1 offspring, both North and South Swedish	+	-

3) Hansson found differences between wild caught animals and F1 offspring, whereas Gustafsson and co-workers found no such differences.

In most respects, the results are consistent from colony to colony. Characters which can be measured both in nature and in laboratory colonies are also similar. This consistency indicates that laboratory animals are not too different from wild ones, and that differences in for example reproduction between colonies from different localities reflect real differences between the localities.

3.2. Pregnancy failure

Several authors report pregnancy failure in *Clethrionomys* females (*C. glareolus*: Westlin & Nyholm 1982; *C. rutilus*: Hoyte 1955, Wallgren et al. 1984, Ims 1985; *C. rufocanus*: Westlin 1982a).

It is not known whether the frequency of pregnancy failure is related to population dynamical events. In *C. rutilus*, Wallgren et al. (1983) and Ims (1985) reported pregnancy failures only in peak years. In *C. glareolus*, on the other hand, evidence of pregnancy failure was seen in all years, even though the density fluctuated violently (Gustafsson unpubl.). Also, Westlin & Nyholm (1982) found similar frequencies of pregnancy failure during spring in cyclic and non-cyclic *C. glareolus*.

The cause most commonly suggested for this pregnancy failure is pregnancy block (Bruce, 1960) caused by contact with non-stud males. In the laboratory, exposure to a non-stud male readily induces pregnancy block in all studied microtine species (Clulow et al. 1982). In the case of *C. rutilus* where pregnancy failure mainly is seen at peak densities (or when *C. rufocanus* reach high densities in the same habitats), pregnancy block seems to be a quite likely explanation.

In *C. glareolus*, on the other hand, pregnancy failure may be part of the normal maturation process. Pregnancy failure is common in two categories of females in this species (Westlin & Nyholm 1982).

1) In overwintered females, before their first pregnancy of the season.

2) In young females, before their first pregnancy.

Young *C. glareolus*, when kept constantly with one male, show low fertility at first mating, and they often mate several times over a few days. After such matings, the histological picture of the failure in the ovary is very similar to that seen after pregnancy failure in the wild (Westlin & Nyholm 1982).

The fertility of young females is a function of age: only 20–25 % of first matings are fertile in 30–50 day old females and 80 % in 100+ day old (Westlin & Gustafsson 1985). Young females seem to need a larger amount of copulatory stimulation than what is normally delivered at a mating. When young females were subjected to mechanical cervical stimulation after mating until satiety, fertility was increased from 29 % to 76 % (Westlin & Gustafsson 1985).

The first matings seem to stimulate the female to reach full fertility much earlier than if not mated. In females, which were allowed to mate with a vasectomized male when 30–40 days old, fertility of matings with normal males betwen 40 and 60 days of age was much higher (63 %) that of similarly aged primiparous females (24 %) (Westlin & Gustafsson 1983).

Physiologically, the infertility seems to be caused by a failure of the mating to initiate the prolactine release necessary to activate the corpora lutea. Prolactine therapy after mating results in activation of the corpora lutea, and full fertility, while progesterone therapy results in full fertility without activation of the corpora lutea (Westlin 1982b).

Acknowledgements. The Oslo-part of this work was supported by the Nansen foundation.

References

Bondrup-Nielsen, S. 1985: An evaluation of the effects of space use and habitat patterns on dispersal in small mammals. — Ann. Zool. Fennici 22:373-383.

Bruce, H. 1960: A block to pregnancy in the mouse caused by proximity of strange males. — J. Reprod. Fert. 1:96-103.

Bujalska, G. 1973: Role of spacing behaviour among females in the regulation of reproduction in the bank vole. — J. Reprod. Fert. Suppl. 19:465-474.

—"— 1975: Reproduction and mortality of bank voles and the changes in size of an island population. — Acta. Theriol. 20:41-56.

Clulow, F. V., Franchetto, E. A. & Langford, P. E. 1982: Pregnancy failure in the red-backed vole, Clethrionomys gapperi. — J. Mammal. 63:499-500.

Fuller, W. A. 1979: Regulation of maturation in young Clethrionomys gapperi. — Ekologiya 2:58-65.

Gustafsson, T. O. 1983: Reproduction and social regulation of sexual maturation in relation to population cyclicity in bank voles, Clethrionomys glareolus. — Unpubl. Ph.D. Thesis, University of Lund.

—"— 1984: Influence of the social environment on sexual maturation of male bank voles, Clethrionomys glareolus. — J. Mammal. (in press).

Gustafsson, T. O., Andersson, C. B. & Nyholm, N. E. I. 1983a: Comparison of sensitivity to social suppression of sexual maturation in captive male bank voles, Clethrionomys glareolus, originating from populations with different degree of cyclicity. — Oikos 41:250-254.

Gustafsson, T. O., Andersson, C. B. & Westlin, L. M. 1983b: Reproduction in laboratory colonies of bank voles, Clethrionomys glareolus, originating from populations with different degrees of cyclicity. — Oikos 40:182-188.

Hansson, L. 1985: Geographic differences in bank voles Clethrionomys glareolus in relation to ecogeographic rules and possible demographic and nutritive strategies. — Ann. Zool. Fennici 22:319-328.

Hansson, L. & Henttonen, H. 1985: Regional differences in cyclicity and reproduction in Clethrionomys species: Are they related? — Ann. Zool. Fennici 22:277-288.

Hoyte, H. M. D. 1955: Observations on reproduction in some small mammals of Arctic Norway. — J. Anim. Ecol. 24:412-425.

Ims, R. A. 1985: The effect of pregnancy failure on the onset of reproduction in sympatric populations of Clethrionomys rutilus and C. rufocanus. — Ann. Zool. Fennici 22:309-312.

Jensen, T.S. 1982: Seed production and outbreaks of non-cyclic rodents in decidious forests. — Oecologia (Berl.) 54:184-192.

Kalela, O. 1957: Regulation of reproduction rate in subarctic populations of the vole, Clethrionomys rufocanus. — Ann. Acad. Sci. Fennicae (A IV) 34:1-60.

Koshkina, T. V. & Korotkov, Y. S. 1975: Regulative adaptation in populations of the red vole (Clethrionomys rutilus) under optimum conditions of its range. — In: Fauna and Ecology of the Rodents 12:5-61. Translated by W. A. Fuller.

Saitoh, T. 1981: Control of female maturation in high density populations of the red-backed vole, Clethrionomys rufocanus bedfordiae. — J. Anim. Ecol. 50:79-87.

Stenseth, N. C. 1977: Theoretical studies on fluctuating populations: an evolutionary approach. — Unpubl. Doctoral Thesis, Univ. of Oslo.

Viitala, J. 1977: Social organization in cyclic subarctic populations of the voles Clethrionomys rufocanus (Sund.) and Microtus agrestis (L.). — Ann. Zool. Fennici 14:53-93.

Wallgren, H., Tähkä, K. & Rosokivi, V. 1984: Reproductive and adrenal functioning in Clethrionomys rufocanus and C. rutilus during one population cycle. — Acta Zool. Fennica 171: 145-147.

Westlin, L. M. 1982a: Sterile matings at the beginning of the breeding season in Clethrionomys rufocanus and Microtus agrestis. — Can. J. Zool. 60:2568-2571.

—"— 1982b: Increased fertility in young primiparous female bank voles, Clethrionomys glareolus, treated with prolacting or progesterone after mating. — J. Reprod. Fert. 66:113-115.

Westlin, L. M. & Nyholm, N. E. I. 1982: Sterile matings initiate the breeding season in the bank vole, Clethrionomys glareolus. A field and laboratory study. — Can. J. Zool. 60:387-391.

Westlin, L. M. & Gustafsson, T. O. 1983: Influence of sexual experience and social environment on fertility and incidence of mating in young female bank voles (Clethrionomys glareolus). — J. Reprod. Fert. 69:173-177.

—"— 1985: Influence of age and of artificial vaginal stimulation on fertility in Clethrionomys glareolus. — J. Reprod. Fert. (In press).

Williamson, M. I. 1972: The analysis of biological populations. — Arnold, London.

Received 11.II.1984
Printed 11.X.1985