THE BANK VOLE
AS EXPERIMENTAL ANIMAL
Second Edition

With Special Focus on its *Stereotypies* and Potential as Model of *Diabetes* and *Seizures*

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Contents.

Preface.

Bank voles have in the past been used as research animals for areas as diverse as predator/prey interactions, population dynamics, effects of photoperiod on reproduction, effects of pollution on their physiology/behaviour, and in many studies concerning prevalence of virus, viral antibodies, bacteria and various parasites. Such studies are still going on in mainly Poland, Russia and Scandinavia.

Their frequent development of captivity- or drug-induced stereotypical behaviours have also been the subject of a few studies. Such stereotypies among bank voles have previously been suggested to model various human psychiatric disorders, and since stereotypies typically develop when animals are maintained under cramped and impoverished conditions, a good model could increase the basic understanding of the development of these stereotypies and their implications.

The topic of this book is basically how to acquire and keep bank voles as research animals, and also how captivity can transform a healthy Danish bank vole into an animal model of type 1 diabetes, reflex seizures, besides this general model of stereotypies. The actual reasons for a sudden development of diabetes, seizures, or stereotypies in a previously healthy (and "normal") organism are still unknown, and without good models I personally doubt these enigmas will be solved anytime soon. The search for new and better animal models will most likely continue for all foreseeable future, but for now, I believe that the bank vole has the potential to make a significant contribution.

The basis for this book is my five years of hand-on experience with bank voles, selected chapters/excerpts from my resultant PhD dissertation [1] and now deleted excerpts from my private homepage (www.clethrionomys.info). The difference between 1st and 2nd edition of *The Bank vole as Experimental Animal* is a couple of minor corrections and I will like to thank Tonny Freimanis for his valuable assistance as copy editor. I hope you will find this book useful for your research.

Bryan Schønecker.

Hareskovby, Denmark, April 2014.

Abbreviations.

3Rs	The principle of Reduction, Refinement, and Replacement.
5-HT	Serotonin (5-hydroxytryptamine).
BB-DP	BioBreeding diabetes-prone rats.
BS	Backward somersaulting; a vole which performs BS.
CIS	Captivity-induced stereotypies.
CNS	Central nervous system (brain and spinal cord).
DA	Dopamine.
DWI	Daily water intake.
F1, F2, F3	1st, 2nd and 3rd generation born in captivity, respectively.
GABA	Gamma-aminobutyric acid.
GAD65	Glutamic acid decarboxylase 65 (molecular weight 65 kDa).
HPA	Hypothalamic-pituitary-adrenal.
IA-2	Tyrosine phosphatase protein ICA512.
IHC	Immunohistochemistry.
JUMP	High-speed jumping; a vole which perform JUMP.
LADA	Latent autoimmune diabetes of the adults.
LV	Ljungan virus.
NMDA	*N*-methyl-D-aspartic acid.
NN	A non-PD and non-STER vole.
NOD	Non-obese diabetic mouse.
OCD	Obsessive compulsive disorder.
P and *P*	Parental generation (wild caught bank voles); Probability value.
PA	Pacing following a fixed route; a vole which perform PA.
PD	Polydipsia (excessive water intake); a vole which is PD.
PDS	A vole(which both exhibit PD and STER.
PD-STER	A PDS vole, which first develops PD, then STER.
PIS	Pharmacologically-induced stereotypies.
PUUV	Puumala hantavirus.
SP	A seizure-prone vole (i.e. at least one observed seizure)
SR	A seizure-resistant vole (i.e. seizures never observed).
SSRI	Selective serotonin reuptake inhibitors.
STER	Stereotypical behaviour; a vole which perform STER.
STER-PD	A PDS vole, which first develops STER, then PD.
T1D and T2D	Type 1 diabetes; Type 2 diabetes.

Chapter 1 - Enter the bank vole.

1.1 Name.

Bank voles (*Clethrionomys glareolus* – Schreber 1780) are known under several local names of which can be mentioned Rødmus (Danish), Skogssork (Swedish), Klatremus (Norwegian), Metsämyyrä (Finnish), "Topillo rojo" (Spanish), Rötelmaus (German), Nornica ruda (Polish) and Campagnol roussâtre (French) [2].

In 2003 it was argued that *Myodes* should properly replace *Clethrionomys* as generic name for red-backed voles [3]. The northern red-backed vole (*Mus rutilus*, Pallas 1779) had according to Carleton, Musser, and Pavlinov indeed been categorised under three generic names: *Myodes* in 1811 by Pallas, *Clethrionomys* in 1850 by Tilesius, and *Evotomys* in 1874 by Coues. However, northern red-backed voles had evidently *also* been assigned as the so-called *type species* (i.e. the actual specimen used to define a taxonomic group) for *Myodes* in 1883 by Lataste; for Evotomys a little later (Carleton et al. mentions an example from 1896), and for *Clethrionomys* in 1928 by Palmer. After that, the vast majority of researchers assigned red-backed voles to the genus *Clethrionomys*, but a few continued to use *Myodes* as the proper name for the genus. The point made by Carleton et al. was that when two or more genera share the same type species, the oldest name must take priority. Ergo, exit *Clethrionomys*, and enter *Myodes* [3].

Musser and Carleton would two years later perpetuate this conclusion in a recognised reference book [4], and the first to respond were (as usual) online thesauruses like e.g. Wikipedia. By 2009, the name *Myodes* had spread to a significant fraction of peer-reviewed papers, and by 2011 the dominance of *Myodes* was almost complete.

The factual premises for Carleton et al.'s conclusions were not discussed by other experts in this particular field until Tesakov, Lebedev, Bannikova, and Abramson did so in 2010 and basically documented that the before-mentioned Coues in fact assigned the brown lemming (*Mus lemmus*) as type species for *Myodes* in 1877. The consequence hereof is that the later assignment by Lataste in 1883 of *Mus rutilus* as type species for *Myodes* is invalid. Ergo, exit *Myodes* and re-enter *Clethrionomys* as rightful name for the genus, including all red-backed voles [5].

1.2 Appearance and measurements.

Adult European bank voles weigh 12-40 g, have a head-body length of 8-13.5 cm and a tail of 3.5–7.2 cm [6]. The tail is usually moderate hairy and two-coloured (light ventrally; dark brown/blackish dorsally), sometimes with a little tuft of slightly longer hairs. The fur is typically light grey/whitish ventrally and in young voles (age 1-3 months) brownish on the flanks and back. The adult colouring, a warm chestnut, typically begins to replace the brownish colourings of sub-adults by the age of two months, and the colour is usually fully developed at age three months (personal observation).

1.3 Placement in the phylogeny.

According to a 2005 taxonomy, red-backed voles are members of the class *Mammalia*, order *Rodentia*, suborder *Myomorpha*, superfamily *Muroidea*, family *Cricetidae*, subfamily *Arvicolinae*, and genus *Myodes* [7]. Musser and Carleton operate with 12 sister-species in this genus (which should now be re-named *Clethrionomys*), and their Latin (common) names are: *M. andersoni* (Anderson's red-backed vole), *M. californicus* (Western red-backed vole), *M. centralis* (Tien Shan red-backed vole), *M. gapperi* (Southern red-backed vole), *M. glareolus* (Bank

vole), *M. imaizumii* (Imaizumi's red-backed vole), *M. regulus* (Korean red-backed vole), *M. rex* (Hokkaido red-backed vole), *M. rufocanus* (Grey red-backed vole), *M. rutilus* (Northern red-backed vole), *M. shanseius* (Shanxi red-backed vole), and *M. smithii* (Smith's red-backed vole) [8]. Viro and Niethammer operated with 29 sub-species plus eight-nine sister-species back in 1982 [6].

I have so far seen five different placements in the taxonomy of these red-backed voles. Since any given taxonomy will be the result of an interpretation of available evidence, it follows that "today's truth" will most likely differ from "tomorrow's", so be prepared to be confused if you consult more than one contemporary taxonomy.

1.4 Distribution and population dynamics.

Species of Clethrionomys are found on all continents of the northern hemisphere. More specifically, the European bank vole is found between 8° W in Great Britain to 90° E in Altai Mountains and from 38° to 68° Northern latitude [6]. The type specimen of *Clethrionomys glareolus* was in fact caught on Lolland (a Danish island) by the German naturalist Johann Christian Daniel von Schreber in 1780 [6].

An online resource such as the "IUCN Red List of Threatened Species" provides a map of its present distribution and list the following countries with a native population of bank voles: Austria, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Macedonia, the former Yugoslav Republic of Moldova, Montenegro, Netherlands, Norway, Poland, Romania, Russian Federation, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, and United Kingdom [9].

Bank vole population densities fluctuate on an annual basis in most of their southern range, whereas the populations in the northern parts of Fennoscandia (Scandinavian Peninsula, the Kola Peninsula, Karelia and Finland) can show multi-annual fluctuations (reviewed in ref. [10]).

It is assumed that Danish bank vole populations show both annual and multi-annual fluctuations [11]. Nielsen provides an example of annual fluctuations in a particular (1 km^2) area of Danish forest (Capture index (May) = (5/250)*100 = 2;

Capture Index September = (116/360)*100 = 32.22), [12]. During his 10-year study on Danish bank vole populations in the mainland (Jutland), Jensen found that the number of bank voles/ha did not exceed a 15-fold difference between May and October (typically 10-60 bank voles/ha; locally up to 125); that mast years (i.e. years with an exceptional production of seeds) always preceded peak densities, and that winter reproduction always preceded such peaks in the populations [13].

I have taken 2-3 daily trips to the forest where I live (Zealand, Danish island) for many years now, and in my time I have experienced some "perfect" vole-years, and also some extremely poor ones. A "perfect" vole-year is also in my practical experience founded the previous year with a mass production of seeds from trees, and here in Denmark that would be from the common beech (*Fagus sylvatica*) and oak (*Quescus robur*). Such a mast year occurs every 3-6 years or so and if the following winter includes a stable and dry snow coverage for some months, this factor will provide protection against both the cold and predators. Result being that when spring breaks, the population will already be large, the predators will be few (it is harder to catch rodents hiding under snow), and food will be plentiful.

The year 2009 was such a mast year in Denmark - the following winter of 2009/2010 was very cold with snow coverage for 3-4 months - and already in march I would see bank voles running around on the forest floor each time I stopped for a minute. When the summer arrived, I could walk into a pine plantation and at any given moment see maybe 4-10 bank voles running freely on the forest floor in plain view. That, I might add, is exceptional in Denmark. Bank voles will in my experience by far prefer to stay in beech forests, presumably due to a better coverage from predators.

However, after a particularly long period (3-4 weeks) with almost constant rain in the mid summer of 2010, the bank voles disappeared completely from sight, and the next time I saw a bank vole in the forest was in late October. That somewhat anecdotic observation illustrates another aspect of the bank vole population dynamics relevant for those who wish to capture them: Bank voles do not appear to tolerate rain very well and if it had rained for maybe 3-4 hours a given day there would usually not be a single vole in the traps the same afternoon, and it would typically take two dry days before they started to appear in the traps again. Without

having examined this issue personally, it is my subjective belief that Danish bank voles easily die of exposure because their fur does not appear to be particularly water repellent, and they can only take so much rain. A paper by Radwan et al. included the information that Polish bank voles would become hypothermic if they were soaked and placed in a wet chamber with a temperature of 23 degrees C for up to 17 minutes (their final body temperature would be 30-34 degrees C), so it seems there is a consensus between my empirical observations and the results presented in Radwan et al.'s paper [14].

To give an example of a particular “poor” vole-year in Denmark, 1995 springs to mind. I started my daily trappings in April with about 150 traps or so - checked them twice daily, repositioned them every few days to find the best habitats for voles, and caught my first bank vole in August.

1.5 Habitat and food.

The bank vole prefers a young forest with ample opportunities for cover (plants, scrubs, bushes, twigs, etc.). It easily climbs threes and bushes to eat buds, shoots, seeds and fruits, and the reason it is considered a pest in Denmark is its habit of also eating the bark of trees, preferably starting in the branches, a little up in the tree. [15]. Most online thesauri will also add that bank voles can eat insects, spiders and fungi, so it would appear to be somewhat omnivorous. Bank voles begin to store supplies of seeds and nuts during autumn, which they keep in extensive borrows, reaching depths of 40-50 cm from the surface [15].

1.6 Parasites, bacteria and viruses in wild bank voles.

Wild-living bank voles can be infected with several different species/strains of parasites, bacteria and viruses of which some are proven zoonoses, and others suspected. I suppose the same must apply to their offspring, at least for some time/generations.

The *Puumala hantavirus* (PUUV) should be of particular concern since it is commonly associated with bank voles throughout Europe and causes a mild form of hemorrhagic fever with renal syndrome (Nephropathia epidemica) [16]. Most of the

cases in Denmark stems from the island Funen where a study found that 14% of the overwintering bank voles were seropositive for PUUV. The study further found that bank voles from the mainland (Jutland) were seronegative [17]. PUUV has long been believed to infect humans through inhalation of aerosols of rodent excreta contaminated with virus [18]. Viral RNA is present in the blood of PUUV-infected bank voles for many months, and infected voles excrete PUUV through their saliva, urine and faeces, particularly in the first couple of months following infection [19]. PUUV-negative bank voles can be successfully infected after nasal inoculation of PUUV-positive saliva, urine and faeces [19]. If the colony includes bank voles with fully developed locomotive stereotypies, those involved in the direct handling of the animals should consider wearing respiratory protection against the tiny dust particles invariably released during such stereotypies.

Another virus, which could be mentioned, is the *Ljungan virus* (LV) [20], or rather the *Tjuonavaggejåkka virus*, as it evidently should have been more properly named instead [21, 22]. This LV virus has been suggested as zoonose relevant to human diseases as diverse as diabetes, heart diseases, sclerosis, spontaneous abortions, sudden infant death syndrome, plus a couple of other diseases [23-32]. However, later works by independent researchers (i.e. researchers without the obvious conflict of interest of assigned inventorship on one or more LV-related patents/-applications) have either found no support for one of these zoonotic relationships (diabetes: see refs. [33-36]), or questioned previous works on various grounds [37-39]. I have in fact only been able to find one review by an independent author expressing the view that the LV might be a source of concern [40].

Wild-caught bank voles can carry ticks (*Ixodes ricinus*) which in turn can be infected with a bacteria (*Borrelia burgdorferi* - a spirochete) which eventually can cause the disease *borreliose* and *tick borne encephalitis*, if transferred to humans [41]. Bank voles can be heavily infected with these ticks [42].

Bank voles can also carry other bacteria and viruses: Fernie & Healing established e.g. that English bank voles could be infected with Campylobacter (*Microaerophilic vibrios*) - a type of bacteria that causes cows to become sterile; provoke sheep to miscarriage, and make pigs dysenteric [43]. Kaplan et al. also examined English voles (*C. glareolus*) along with the Skomer vole (*C. glareolus skomerensis*) and

discovered antibodies against *Ectromelia* (Mouse pox) virus; *Louping ill virus*; *Reovirus III*; *Lymphocytic choriomeningitis virus,* and *Encephalomyocarditis virus*. The vast majority of these antibodies were directed against the *Pneumonia Virus of Mice* and the *Sendai virus* [44].

1.7 Breeding season in the wild.

In the wild, breeding is normally restrained to the spring, summer and autumn with the longest breeding season in the southern distribution area and the shortest in the northern. The breeding season in e.g. France lies between February and October [6]. In Denmark the breeding will typically take place from April to September, but should food be plentiful, as following a mast year, breeding can continue throughout the winter [13, 45].

1.8 Reproduction and longevity in captivity.

Danish bank voles, when maintained indoor as single pairs in moderately enriched cages, delivered pups all year round (personal observation), paralleling a much earlier English study which showed that British bank voles also can breed year-round when maintained in large cages in open air under conditions approaching the wild state [46]. Christiansen & Døving provide an, in my opinion, both vivid and accurate description of the mating behaviour of bank voles [47], but where the authors felt "*the most conspicuous feature of the mating behavior was the* [short - a few seconds] *duration*", I find the most conspicuous feature to be the way an inexperienced naïve male would sit on its hind legs after having dismounted a female for the first time, and then look down for maybe 2-4 seconds on its pelvic region with what I can only describe as "surprised interest".

Bank voles are so-called "induced ovulators", meaning that ovulation follows coital stimulations [48, 49], but ovulation can be induced, too, by the presence of males in the same cage as the females [50]. Towards the end of pregnancy the adrenals increase 3-4 times in weight [51]. According to Chitty and Clarke (1968) and Jorné-Safriel (1968), "*The growth is confined to the adrenal cortex and…the inner zone of the cortex widens considerably*" (both papers cited in Gustafsson & Anderson,

[52]). This hypertrophy is triggered by the act of mating itself, i.e. not caused by ovarian hormones, and it has been suggested that the triggering mechanism should be a neuroendocrine reflex [52]. However, pregnancy itself seems to contribute to the adrenal hypertrophy since 6-day pregnant females had significant heavier adrenals than mated, but non-pregnant, females [52].

Bank voles weigh approximately 2 grams at birth [53], and earlier studies have reported litter-sizes of 3.6 pups/litter (range 1-10 pups/litter; 2862 pups in 728 litters; outbred polish bank voles; Buchalczyk (1970) [54]) and 4.3 pups/litter for primiparous females, increasing to 5.3 pups/litter for multiparous females (range 1-10 pups/litter; 2844 pups in 827 litters; inbred Swedish bank voles; Gustafsson et al., (1980) [55]). My own results in relation to pups/litter, based on three outbred colonies of 1307 Danish bank voles in total, are quite comparable to the results from Buchalczyk and Gustafsson et al.

The largest litter-sizes are delivered at ages 6-14 months, whereafter the litter-sizes gradually decrease [54]. It has long been noted how lactation prolongs the gestation time of subsequent litters due to a delayed implantation of the blastocyst in the uterus [53, 56].

The average length of pregnancy is 18.3 days for primiparous females and 22 days among lactating females [55]. Such post-partum matings (successful mating immediately after the female had given birth, or during the first 1-2 days) is indeed common among red-backed voles, and Clarke & Hellwing [57] described this phenomenon very well using English bank voles, with a distribution of data, which are very similar to my own. They found that 50% of the lactating females were mated 1-2 days after the birth of their litter where Morrison et al. [58], on a previous occasion had found that 57% of their Northern red-backed voles were mated post-partum (exactly during which time-interval was not specified).

Buchalczyk [54] found that the mean captive lifespan of female and male [Polish] bank voles forming reproducing breeding pairs was 525 days (17.3 months) and 683.2 days (22.5 months), respectively. Non-reproducing pairs lived shorter (mean female lifespan was 309.8 days (10.2 months); mean male lifespan was 417.2 days (13.7 months)), and both females and males, when maintained in single-sex cages with 5-7 other same-age voles, lived the shortest (females for 162 days (5.3 months);

males for 208.5 days (6.9 months)). Lastly, males lived significantly longer than females with a maximal lifespan of 50, respective 45, months [54].

1.9 Diurnal activity in captivity.

Danish bank voles measured in a Danish animal facility during the summer exhibited a polyphasic activity pattern, with one period of increased activity centred around 1000-1200 hours, and a relatively more intense period of activity centred around 2200-2400 hours [59]. These periods of increased activity typically lasted 3-4 hours, and in between there was a steady, overall, decrease in activity. In my personal experience there would typically be brief periods in between, lasting from 15 minutes to a half hour where the bank voles seem to synchronize their activity. These synchronizations did not appear to be related to any particular outside disturbances, and I must emphasize that I am simply conveying my personal impression.

A previous study by Ödberg (using bank voles from Belgium, presumably descendants from imported *C. glareolus britannicus*, originally caught around Edinburgh, [60]) was the first to analyse the activity patterns of bank voles, and all in all Ödberg's results, as presented in his resultant paper [61], are about the same as for the Danish bank voles.

Chapter 2 - How to get your bank voles.

2.1 Legal matters.

At least here in Denmark, virtually all wild living mammals, birds, reptiles and amphibians are protected against being collected or killed, including some species of plants and fish. There are of course some exceptions for those with a licence to kill (albeit not collect), but unless you are affiliated with a research organisation of sorts and have the right permits, you can forget about collecting bank voles for studies. You will typically have to...

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End of free sample