

Esa Koskela

Reproductive Trade-offs
in the Bank Vole



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ABSTRACT

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Diss.

The aim of this thesis was to develop an understanding of reproductive trade-offs using the bank vole *Clethrionomys glareolus* as a model organism. The studies were mainly carried out as experiments in large outdoor enclosures and a laboratory colony of bank voles was used in quantitative genetic analyses of life-history traits. Litter size manipulations were performed to study the trade-off between number and quality of offspring and reproductive costs. Neither enlarging nor reducing litter size increased the number of offspring entering the breeding population, and the litter enlargements decreased offspring body mass at weaning. In male offspring lower body mass was maintained over winter until next spring. Future fecundity or survival of mothers was not affected by litter size manipulations. To study ecological factors affecting reproductive success, food resources and breeding density during nursing were manipulated together with litter size in two experiments. The weaning success of females was improved both in lower density and with supplemented food. However, when offspring in enlarged litters suffered from lower mass independent of density, with supplemented food litter enlargements did not result in lower weight of weanlings. Spacing behaviour of females was dependent on the phase of reproductive cycle, food availability and breeding density. The results from both laboratory and field experiments indicate that size at birth predicts breeding success: larger female offspring matured earlier than smaller ones. Heritability estimates were high both for litter size ($h^2 \pm se: 0.68 \pm 0.22$) and body mass at birth (0.77 ± 0.21) indicating high amount of genetic variance in these traits. Both phenotypic and genetic correlations were negative between the number and size of offspring indicating antagonistic pleiotropy between the two traits. The results suggest that resource allocation between the number and size of offspring is an important fitness component for bank vole females. This allocation seems to be determined mainly during pregnancy. In addition, the amount of food available is an important factor determining the reproductive success of bank vole females.

Key words: *Clethrionomys glareolus*; genetic correlation; heritability; mammals; offspring size; reproductive costs; reproductive effort; trade-off.

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List of original publications

This thesis is based on the following articles, which will be referred to by their Roman numerals. I have written papers I - IV and performed most of the work. I have performed a significant proportion of the work in gathering data and writing paper V.

- I Koskela, E., Jonsson, P., Hartikainen, T. & Mappes, T. 1998. Food limitation on reproductive success: an experiment in the bank vole *Clethrionomys glareolus*. Proc. R. Soc. Lond. B. (In press).
- II Koskela, E., Mappes, T. & Ylönen, H. Experimental manipulation of breeding density and litter size: effects on reproductive success in the bank vole. Manuscript (Submitted).
- III Koskela, E., Mappes, T. & Ylönen, H. 1997. Territorial behaviour and reproductive success of bank vole *Clethrionomys glareolus* females. J. Anim. Ecol. 66: 341-349.
- IV Koskela, E. 1998. Offspring growth, survival and reproductive success in the bank vole: a litter size manipulation experiment. Oecologia (In press).
- V Mappes, T. & Koskela, E. Genetic basis of the trade-off between offspring number and quality. Manuscript.

1 INTRODUCTION

1.1 General introduction

Natural selection should cause individuals to allocate their reproductive effort between and within reproductive attempts in a way that maximizes their lifetime reproductive success. Optimal reproductive effort at a given breeding attempt is regulated by several trade-offs between life-history traits that are imposed by various constraints. The study of the evolution of life-histories aims at determining these trade-offs and their dependence on the state and ecological environment of an individual (Cole 1954, Gadgil & Bossert 1970, Hirshfield & Tinkle 1975, Stearns 1976, 1992, Roff 1992).

According to the Principle of Allocation (Levins 1968) individuals have a limited set of resources (e.g. energy) to invest in different processes. A trade-off results when resources are allocated between traits and a beneficial change in one trait is linked to a detrimental change in another. Two major fitness trade-offs have been recognised in the life-history theory. First, the trade-off between number and quality of offspring: as number of offspring increases, the quality of an individual offspring decreases (Lack 1947, Smith & Fretwell 1974). Second, the trade-off between current and future reproduction (cost of reproduction): an increase in the energy used for the present breeding attempt results in a decrease of energy available to other functions, such as maintenance or future breeding (Williams 1966, Bell 1980). All other trade-offs can be considered as examples of these two major trade-offs (Lessels 1991).

A major tool used in the study of life-history evolution is optimality theory (Gadgil & Bossert 1970, Schaffer 1983, Parker & Maynard Smith 1990). It concerns the trait combination that maximizes life-time fitness and assumes natural selection to be capable of producing the optimal phenotype. Within the optimality theory, several different approaches have been used to study trade-offs (Bell & Koufopanou 1986). The simplest method is to measure naturally occurring variation between traits at phenotypic level. However, this approach does not take into account the fact that trade-offs may be masked by among

individual variation in acquisition of resources. This is solved in the second approach, the use of experimental manipulations, where individuals are randomly allocated to different treatment groups. The third approach is to calculate genetic covariance among traits (genetic correlations). The validity of these methods has itself been the subject of intensive debate (e.g. Reznick 1985, Partridge & Harvey 1985, Pease & Bull 1988, Partridge 1992, Reznick 1992a,b). The general conclusion from this discussion is that experimental manipulations provide a good basis for understanding the mechanisms involved in a trade-off, whereas a genetic analysis reveals its evolutionary significance. In other words, while optimization theory can be successful in predicting which combination of traits should evolve, it does not provide us with an understanding of the evolutionary processes. Quantitative genetic theory provides tools for this purpose (Fisher 1930, Lande 1982). Methods in quantitative genetics enable the estimation of heritabilities and genetic covariances between traits under study, and allow predicting how the traits will evolve (Roff 1997, Lynch & Walsh 1998).

Although the life-history theory works as a general framework for the study of life-histories, the experimental evidence for several main hypothesis originates from a narrow range of organisms. For instance, numerous studies have examined reproductive trade-offs in hole-nesting altricial birds whereas testing the same hypotheses in mammals has been scarce. This is the case e.g. with the studies of optimal brood size (for reviews, see Klomp 1970, Murphy & Haukioja 1986, Godfray et al. 1991, VanderWerf 1992) and cost of reproduction (for reviews, see Bell & Kofopanou 1986, Lindén & Møller 1989, Roff 1992, Stearns 1992). This is unfortunate, because many hypotheses that are difficult to study in certain species are more easily studied in others. Further, testing the generality of the hypotheses of life-history theory would require experimental studies from a broad range of organisms.

1.2 Aim of the thesis

The aim of this thesis was to develop an understanding of reproductive trade-offs using the bank vole *Clethrionomys glareolus* as a model organism. Using both optimization and quantitative genetic approach my aim was to study the determinants of reproductive success and reproductive effort in individual bank vole females. The two main life-history traits under study were litter size and offspring size.

In the first two papers I examined the effects food availability (I) and density of breeding females (II) on reproductive success of nursing females. In the third paper (III) I investigated how territorial behaviour of breeding females was related to the reproductive cycle, and whether it affected the reproductive success of females. The last two papers concentrated on the trade-off between number and quality of offspring. I examined (IV) intergenerational trade-offs between offspring number and their future performance. In the fifth paper I studied (V) if offspring size at birth indicates quality and whether phenotypic

trade-off between offspring number and size was genetically determined. In this last paper I also examined whether the phenotypic variation in litter size and offspring size had a genetic basis. Reproductive costs as possible determinants of reproductive effort were addressed in studies I, II and IV.

2 METHODS

2.1 The species

The bank vole (*Clethrionomys glareolus* Schreber 1780) is a common rodent in Palearctic region. In the study area in central Finland bank voles inhabit various environments from bushy fields to forests. Breeding season lasts from late April to September and individuals usually overwinter only once. Females give birth to up to four litters during the breeding season the litter size ranging from one to ten (usually 4 - 8) (T. Mappes and E. Koskela, unpublished). Pregnancy lasts for 19 - 20 days (in lactating females 21 - 24 days) and pups are weaned before the age of three weeks. Reproducing bank vole females are territorial while male home ranges are larger and overlapping (e.g. Mazurkiewicz 1971, Bujalska 1973, III). Possession of a territory is a prerequisite for breeding in *Clethrionomys* females (Kalela 1957, Bujalska 1973, Kawata 1987). The mating system in the bank vole is considered to be promiscuous (Sikorski & Wójcik 1990). Population densities have been found to fluctuate in 3 - 5 year cycles in northern Fennoscandia (Hansson & Henttonen 1988, Korpimäki et al. 1991). However, during the years of this study (1994 - 1997), population densities in the study area showed fluctuations typical for non-cyclic populations (T. Mappes & E. Koskela unpublished).

Bank voles breed well in enclosures and in laboratory conditions, they have a good trappability and are not sensitive to disturbance e.g. when handled. They do not recognise their pups from foreign ones which enables litter size manipulations and cross-fostering (Mappes et al. 1995). Consequently, the bank vole is a suitable study species for both laboratory and field experiments. The individuals used in the studies were captured around the study site (I - IV) or were laboratory-born descendants of wild bank voles (V).

2.2 The study site

The studies were carried out at Konnevesi Research Station in central Finland (62°37'N, 26°20'E). Parts of the work were conducted at the Experimental Animal Unit of the University of Jyväskylä. In the laboratory the animals were housed in standard mouse cages with wood shavings and hay as bedding. The colony was maintained under 18L:6D photoperiod and food (rodent chow) and water were available *ad libitum*. Experiments I - IV (partly V) were carried out in eight 0.25 ha outdoor enclosures founded on a relatively homogenous field. The fences of the enclosures were constructed by embedding a 1.5 m galvanised metal sheet into the ground to a depth of about 0.5 meter, giving the wall height of one meter. The vegetation in enclosures consisted mainly of grasses (e.g. *Alopecurus pratensis*, *Phleum pratense*, *Elymus repens*, *Deschampsia* spp., *Poa* spp.) and herbs (e.g. *Ranunculus acris*, *Hypericum maculatum*, *Geum rivale*, *Alchemilla* spp., *Trifolium* spp.). There were also some saplings of *Alnus incana* and *Salix* spp. The fences not only enclosed the experimental populations but also prevented the access of small mustelid predators (the least weasel *Mustela nivalis* and the stoat *M. erminea*) to the enclosures.

2.3 Monitoring of individuals

The voles were monitored in the enclosures using live trapping. Twenty-five Ugglan Special multiple-capture live traps were distributed in each enclosure in a 5 x 5 array with 10 meters between the trap stations. During a trapping period the traps were normally checked ten times, twice a day (morning and evening) for five days. In this thesis home range was defined as a foraging area, usually overlapping, whereas territory is part of home range and mutually exclusive apart from other females. Territory could also be considered as a breeding area. Home ranges were calculated by using minimum convex polygons centred on arithmetic mean (Kenward 1987). Nearest neighbour distance was calculated as the distance between the activity centers of neighbouring females. Home range overlap was measured as the proportion of total trap sites in the home range of each female visited also by other females (Ims 1987).

2.4 Litter size manipulation (I, II, IV, V) and cross-fostering (I, II, V)

Maternal effort during nursing was manipulated by changing the original litter size. Because prenatal environment (e.g. intrauterine position) and mother quality may greatly affect offspring phenotype especially in mammals (for reviews see vom Saal 1981 and Clark & Galef 1995), cross-fosterings were

performed to randomise the prenatal maternal effects on performance of pups. Litter manipulations and cross-fosterings were performed within two days after birth. Pups were weighed, sexed (according to the length of the anogenital distance) and individually marked. In litter size manipulations litters of different original size were assigned randomly to two treatment groups: enlarged litters, where two pups were added and to control litters, where the original litter size was not changed. In two studies (IV, V) two pups were removed from the original litter size as a third litter size manipulation category. In cross-fostering all pups in a litter were replaced and when possible, the sex ratio of mothers was not changed. According to earlier experiments, the growth or survival of pups do not differ between the female's own pups and cross-fostered pups (Mappes et al. 1995, E. Koskela et al. unpublished data).

2.5 Food limitation and reproductive success (I)

To study whether food availability limits reproductive success, food resources (control vs. supplemented) and brood size (control vs. +2 pups) of lactating females were manipulated during nursing period in the enclosures. Further, the possible effects of supplemental food on growth and subsequent breeding of females were studied.

Females gave birth in the laboratory. After litter size manipulations, females with their litters were transferred to the enclosures. Four females were assigned to each enclosure, two nursing a control litter and two an enlarged litter. Two separate replicates were carried out, in both replicates four enclosures were provided *ad libitum* with supplemental food (laboratory rodent chow, Labfor R36) while another four served as controls without extra food. Supplemental food was provided in wiremesh feeders at every trap station. Feeders were set at the time the females and pups were released to the enclosures and removed before the pups reached the trappable age. Before parturitions of subsequent litters, females were removed from enclosures. Female body size after nursing and subsequent litter size were determined in the laboratory. The offspring nursed and weaned in the enclosures were captured, weighed and measured (head width) at 30 days of age.

2.6 Breeding density and reproductive success (II)

The effects of density of breeding females on number and mass of weaned offspring were studied by manipulating simultaneously the litter size (control vs. +2 pups) and breeding density (high vs. low) of nursing females. In the beginning of the experiment six females were released to each enclosure. After a seven day habituation period three mature males were introduced into each enclosure. Before parturitions, females were removed from the enclosures and

housed in standard breeding cages in the laboratory until they gave birth. Litters were manipulated and cross-fostered within two days after birth (see 2.4). After the manipulations, females and their litters were returned to enclosures. There were high density enclosures with six females in each, three nursing control and three enlarged litters, and low density enclosures with two females in each, one female nursing a control and one an enlarged litter. The spacing dynamics of individuals were monitored twice during the experiment: 1) when females were in late pregnancy of their first litters and 2) after litter size and density manipulations when females were nursing their young. At 30 days of age weanlings were captured and weighed. Before the possible births of second litters females were removed from the enclosures to the laboratory to determine the characteristics of subsequent breeding (litter size, pup mass at birth, postpartum mass of mothers).

2.7 Territorial behaviour (III)

Territorial behaviour of females was studied by estimating home range size, home range overlap and behaviour during different phases of reproduction. The effect of these factors on female reproductive success was also investigated. The five trapping periods were classified as follows: 1) non-pregnant females, 2) early pregnancy, 3) late pregnancy, 4) nursing period and 5) the period when weanlings were recruited to the population. The behaviour of territory owner females against intruder females was determined four times, once for each trapping period during the last two days of trapping. Behavioural trials lasted 10 minutes and were carried out in small arenas (50 x 60 cm) with wiremesh floors and Plexiglas front walls. The arenas were placed directly on the ground at the trap station nearest to the activity center of the owner female. Following behaviours were recorded (following Ims 1987): amicable behaviour, approaching, freezing, avoidance, fleeing, threat and attacks. The reproductive success of females was determined as litter size and number of weaned offspring.

2.8 Intergenerational trade-offs (IV)

Intergenerational trade-offs (Stearns 1989) were studied by manipulating the litter size and monitoring offspring performance until their first breeding season. There were three litter size manipulation groups: reduced (-2 pups), control (two pups exchanged) and enlarged (+2 pups) litters. Mothers and their manipulated litters were released to the enclosures in late summer and they were monitored until the following breeding season in next spring. By studying the mothers' over winter survival, also intragenerational trade-offs were addressed.

2.9 Genetic correlation between the number and quality of offspring (V)

The phenotypic trade-off between number and size of offspring (size measured as body mass and head width at birth) was determined separately for bank voles originating from laboratory and field populations. For genetic analyses females were bred in the laboratory. After parturitions pups were counted, weighed and measured. Cross-fosterings were performed to randomise prenatal maternal effects (see 2.4). Heritabilities of the litter size and offspring size at birth were estimated from mother-daughter regressions. Genetic correlations were calculated from mother-daughter covariances (Falconer and Mackay 1996).

To manipulate offspring size at weaning, litter size manipulations were performed (see 2.4). The quality of different sized females was studied by determining the maturation age both in the laboratory and in the field. In the laboratory female offspring were mated repeatedly from the age of 40 days until they gave birth for the first time. In the field experiment 56 females (mean age of 53 days, from control litters) were randomly selected and released to the enclosures. After a one week habituation period, three mature males were introduced to each enclosure. Before parturitions, all females were simultaneously removed to standard breeding cages in the laboratory where they were checked twice a day for parturitions.

3 RESULTS AND DISCUSSION

3.1 Ecological factors affecting reproductive success

3.1.1 Food resource limitation (I)

The results suggest that the weaning success of bank vole females is limited by food availability. In general, when females were provided with extra food, they weaned larger (body mass and head width) offspring than control females. Enlarging the litter size decreased the body mass and size of offspring at weaning (as in Mappes et al. 1995). However, when food was supplemented, offspring body mass was unaffected by litter enlargement. This indicates that the nursing effort of mothers (i.e. the amount of milk for pups) was limited by food availability. Female body size was also affected by extra food: supplemented females grew bigger than unsupplemented ones during nursing. Home ranges of females with supplemental food were smaller than those of control females (P. Jonsson, T. Hartikainen, E. Koskela & T. Mappes, unpublished).

In agreement by Lack's (1948) argument, the results give experimental evidence that the energy requirements of lactating females pose an important constraint on the number of offspring which can be raised successfully. This view agrees with earlier evidence from birds (review in Martin 1987), for which limiting food resources have been widely accepted as one of the most important factors affecting reproductive success. Availability of food resources during nursing may also affect future reproductive success because females can use more energy for somatic growth.

3.1.2 Density of breeding females (II)

Density had clear effects on reproductive success of females: mothers nursing their young in low density enclosures weaned significantly more offspring than mothers in high density enclosures. Mother's failure to successfully wean any

offspring was more common in high density treatment, whereas litter manipulation or mother body mass did not affect weaning success. Litter enlargements did not increase the number of weanlings per female, and offspring from enlarged litters suffered from lower body mass compared to offspring from control litters. This was true independent of density treatment: even though the mothers increased the size of their home ranges when breeding density was not saturated, it did not result in better quality (i.e. heavier) weanlings. This indicates that density-dependent factors were not limiting the nursing effort of bank vole mothers.

3.2 Territorial behaviour and reproductive success (III)

The spacing pattern of females from sexual quiescence to nursing developed towards strict breeding territoriality. At the same time female home range size and home range overlap decreased, the nearest neighbour distance increased. Further, in the behavioural trials the number of attacks increased and amicable behaviour decreased during the reproductive cycle. Female home range size correlated positively with litter size and there was a tendency for more aggressive females to have larger home ranges. These results indicate a possible link between female behaviour, home range size and reproductive success.

In this study (III), the pup-defence (Wolff 1993) and food-defence (Ostfeld 1985) hypotheses as explanations of territoriality in female mammals were also investigated. At the time of parturition female home range size (foraging area) was at its smallest but territory size (breeding area) at its largest. However, exclusiveness of home range (measured as home range overlap and nearest neighbour distance) did not explain the variation in number of weanlings. The fact that females were most aggressive when pups were present does not demonstrate whether they were protecting food resources or pups. The results give evidence both for and against the food- and pup-defence hypothesis and suggest that they are not necessarily mutually exclusive.

3.3 Costs of reproduction (I, II, IV)

None of the studies in this thesis were specifically designed to study reproductive costs, i.e. the effects of current breeding effort on future fecundity or survival of mothers. However, the litter size manipulations conducted in three studies (I, II, IV) enabled assessing costs of reproduction. The results of paper IV indicated that litter enlargements did not affect mothers over winter survival. This was supported by papers I and II, in which there were no obvious differences in mortality rates of mothers randomly allocated to different litter manipulation groups. The size of mothers, measured as head width (I) or body mass (II), was not significantly affected by litter enlargements.

Probability and characteristics of subsequent breeding in relation to litter manipulation were studied in papers I and II. No clear evidence was found for fecundity costs. The probability of subsequent breeding was not lower in females nursing enlarged litters (I, II), nor were there any differences in the size of subsequent litters (I, II) or pup mass at birth (II).

Taken together, the studies of this thesis did not provide evidence for survival or fecundity costs for mothers. However, even though this result is supported by other experiments performed in mammals (Hare & Murie 1992, Mappes et al. 1995), there are matters that should be taken into account when reporting that costs do not exist. First, brood enlargements do not necessarily induce parents to increase their effort (review in Lessels 1993, Moreno et al. 1995, Tolonen & Korpimäki 1996) and it could give an apparent impression that costs do not exist when they actually do. However, in our laboratory experiments, bank vole females have increased their pup defence activity (P. Juutistenaho, E. Koskela & T. Mappes, unpublished) and foraging (E. Koskela, T. Mappes & T. Virkki, unpublished) when their litter sizes have been artificially increased. This indicates that bank vole females adjust their parental effort according to offspring number and/or size. Second, considerable sample sizes are needed for reliable statistical analyses, for example, of the probability of subsequent breeding (e.g. Graves 1991, Roff 1992). This is possible to take into account by providing the analyses with power values and, if necessary, by gathering more data. Third, environmental conditions may affect manifestation of reproductive costs (Tuomi et al. 1983, Jönsson 1997). In paper I, litter enlargements did not decrease the body mass of weanlings when females were provided with extra food. As the reproductive costs have often been found to appear as offspring trade-offs rather than as parent trade-offs (Lindén & Møller 1989, Roff 1992, IV), results suggest that manifestation of reproductive costs as offspring quality may depend on resource availability.

3.4 Trade-off between number and quality of offspring

3.4.1 Intergenerational trade-offs (IV)

Litter size manipulation did not seem to affect the reproductive success of bank vole females. There were no differences in the number of reproducing female offspring or their litter size between mothers of different manipulation groups. Neither enlarging nor reducing litter size increased the number of male offspring entering the breeding population. Further, the male offspring from enlarged litters were lighter, which might affect success in male-male competition for females, and thus reproductive success (e.g. Sheridan & Tamarin 1988). These results support the individual optimization hypothesis (Morris 1985, Pettifor et al. 1988) which states that the original brood size of parents maximizes the recruitment rate of offspring. Thus, an intergenerational trade-off seems to exist between the reproductive effort of mothers in one year and the reproductive success of their offspring in the following year.

The results further indicated that weaning mass affects long-term survival of females, but appears to have no obvious effects on survival of males. Independent of the manipulation group the heaviest female offspring - that were also the "strongest" - had the best chances of survival. These results may indicate that even though litter enlargements decreased body mass of female offspring, this did not change the original underlying effect of body mass at birth (or other brood traits, Tinbergen & Boerlijst 1990) on their subsequent performance.

3.4.2 Genetic basis (V)

The negative phenotypic correlation between litter size and offspring size found both in the laboratory and the field populations seems to have a genetic basis (V). Heritability estimates for litter size and offspring size as well as phenotypic and genetic correlations between the two traits (from the laboratory population) are presented in table 1. Both the laboratory and the field results showed the importance of size at birth for future breeding success: female offspring that were larger at birth matured earlier than smaller females. Thus size at birth seems to be an important fitness component. The body mass of weanlings in enlarged litters was lower and in reduced litters higher than that of control litters. However, size at weaning did not affect maturation age of female offspring.

TABLE 1 Heritability estimates (h^2) for litter size, offspring body mass and head width and phenotypic (r_p) and genetic correlations (r_A) between these traits. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. n_1 = number of mother-daughter pairs, n_2 = number of mothers.

	$h^2 \pm \text{s.e.} (n_1)$	$r_p (n_2)$	$r_A \pm \text{s.e.} (n_1)$
Litter size	$0.68 \pm 0.22 (89)$ **		
Body mass	$0.77 \pm 0.21 (78)$ ***		
Head width	$0.51 \pm 0.21 (73)$ *		
Litter size vs. body mass		$-0.47 (104)$ ***	$-0.54 \pm 0.16 (78)$
Litter size vs. head width		$-0.47 (102)$ ***	$-0.30 \pm 0.27 (73)$

The study gives novel evidence for the evolutionary trade-off between number and quality of offspring. As litter size can be considered as an important fitness related trait, its high heritability estimate is somewhat surprising (Gustafsson 1986, Mosseau & Roff 1987). High genetic variance in litter size may, at least partly, be explained by negative genetic correlation between number and size of offspring. The observed magnitude of the genetic correlation is characteristic for life-history traits in general (Roff 1996). The results also suggest that the optimal allocation between number and size is determined during pregnancy rather than during lactation. This is contradictory to results from altricial birds, in which the trade-off is mainly determined during the time when parents feed their nestlings (Lack 1947, Smith et al. 1989, Siikamäki 1998).

4 CONCLUSIONS

The results of this thesis provide evidence for the trade-offs determining the reproductive characteristics of the bank vole. An important fitness component in females is the allocation of resources between the number and size of offspring. The life-history problem is to find the optimal allocation of resources between offspring number and size per breeding attempt which maximises fitness. In the bank vole this allocation seems to be mainly determined during pregnancy. However, litter and offspring size are presumably constrained by mechanical factors (e.g. size of pelvis and reproductive tract). In addition, ecological (especially food) and physiological (e.g. body mass) factors further influence the optimal allocation between the two traits. Finally, the allocation between number and size is constrained by the phenotypic and genetic trade-offs between traits. Thus, it seems that in the bank vole optimal offspring (litter) size can not evolve without simultaneous covariation with litter (offspring) size.

Of the ecological factors studied, both food availability and density of breeding females affected weaning success but only food affected the quality of offspring. Consequently, food seems to be the most important ecological factor determining the reproductive success of bank vole females. This further suggests that the optimal litter size would be higher in better food conditions. That food availability seems to be so important for the reproductive success in bank voles is in agreement with evidence from passerine birds, in which the capacity of parents to feed their nestlings is considered to be the major determinant of clutch size (Lack 1947, Lundberg & Alatalo 1992).

Costs of reproduction do not seem to belong to the major determinants of reproductive effort. This could be partly due to the fact that in mammals and most birds survival of offspring early in life is largely dependent on survival of parents. In such conditions, females should not increase their parental effort to a level where survival costs would arise (Tuomi 1990).

Trade-offs between reproductive traits may be influenced by on the spacing behaviour of the species. Spacing behaviour is, however, clearly dependent on reproductive cycle: from sexual quiescence to lactation spacing pattern develops towards strict breeding territoriality. Females with access to

richer food resources have smaller home ranges than control females, whereas home range size does not depend on litter size. Further, territory size in bank voles is inversely dependent on density of breeding females. This plasticity in spacing behaviour reflects the costs and benefits of territory defence in different physiological and environmental settings.

A trade-off between number and quality of offspring is one of the main assumptions in the life-history theory. It is also the main basis of the recent models predicting optimal reproductive allocation in vertebrates (Charnov and Downhower 1995). However, previous evidence of the genetic basis of this trade-off is almost totally lacking (Roff 1992). Thus, an important result of this thesis is the genetic basis of the trade-off between offspring number and quality in the bank vole. The benefits of larger size at birth seem to manifest in the earlier age at first reproduction. In general, breeding at an early age should be beneficial when the population density is increasing (Lewontin 1965). This is typical in non-cyclic small rodent populations, where densities increase through the breeding season.

In conclusion, it seems that the same factors that are important for reproduction in other taxonomic groups play an important role also in the bank vole. Small mammals are well suited for experimental study of life-history evolution. This is demonstrated especially in the possibility to estimate the genetic basis of the traits in question, and to study the trade-offs both in the laboratory and in the semi-natural field conditions in outdoor enclosures. Future work will show how well the results apply to mammals in general.

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Yhteenveto

Lisääntymisen allokaatiokustannukset metsämyyrällä

Väitöskirjatyöni kuuluu elinkiertojen evoluutiota tutkivaan tieteenalaan. Se tutkii eliöiden lisääntymisen sopeutumista ympäristöön luonnonvalinnan periaatteen pohjalta. Mitattavia elinkierron piirteitä kutsutaan elinkierto-ominaisuuksiksi. Tutkimuksessani keskityin selvittämään erityisesti kahden keskeisen elinkierto-ominaisuuden, poikuekoon ja poikasen koon, määräytymiseen ja muunteluun vaikuttavia tekijöitä. Tutkimuslajina oli Suomen yleisin nisäkäs metsämyyrä (*Clethrionomys glareolus*).

Elinkiertoteorian keskeisen periaatteen, allokaatiohypoteesin, mukaan yksilön käytettävissä olevat resurssit eli voimavarat ovat rajalliset, ja ne voidaan jakaa eri elintoimintojen kesken eri tavoin. Periaatteen mukaan resursien lisätty kohdentaminen johonkin tiettyyn toimintaan tai ominaisuuteen johtaa kustannukseen samoista resursseista kilpailevissa toiminnoissa (= allokaatiokustannus eli trade-off). Optimointimallien avulla pyritään ennustamaan, mitkä elinkierto-ominaisuuksien muodostamat elinkiertotaktiikat yleistyvät erilaisissa ympäristöissä. Menetelmä on osoittautunut tehokkaaksi tutkittaessa niitä mekanismeja, jotka määräävät taktiikoiden optimaalisuuden yksilöiden ilmiänsuun tasolla. Optimointiteoria olettaa valinnan pystyvän tuottamaan optimaalisen elinkiertotaktiikan omaavan yksilön. Tämä on kuitenkin mahdollista ainoastaan, jos piirteissä on geneettistä muuntelua. Kvantitatiivinen genetiikka tutkii geneettisen muuntelun osuutta kvantitatiivisissa ominaisuuksissa, joita ovat esimerkiksi massa tai poikuekoko. Väitöskirjatyössäni sovellettiin sekä optimointiteorian että kvantitatiivisen genetiikan tutkimusmenetelmiä. Valtaosa työstä tehtiin 0.25 hehtaarin laajuudessa ulkotarhoissa maastosta pyydetyillä yksilöillä, ja kvantitatiivisen genetiikan mittaukset tehtiin laboratorio-populaatiossa.

Naaraan alkuperäisen lisääntymispanostuksen muuttaminen poikuekoko manipuloimalla ei kasvattanut vieroitettujen poikasten määrää. Poikuekoon kasvattaminen alensi kuitenkin poikasten vieroituspainoa, joka saattaa ennustaa huonompaa lisääntymismenestystä ja/tai hengissäsäilyvyyttä tulevaisuudessa. Emojen hengissäsäilyvyyteen tai tulevaan lisääntymiseen lisääntymispanostuksen muuttamisella ei ollut vaikutusta. Nämä tulokset viittaavat metsämyyränaaraan optimoivan lisääntymispanostuksensa tasolle, joka maksimoi hyvälaatuisten vieroitettujen poikasten lukumäärän heikentämättä omaa elinkykyä tai lisääntymismenestystä tulevaisuudessa.

Lisääntymismenestykseen vaikuttavista ekologisista tekijöistä tutkittiin erikseen ravinnon sekä lisääntymistiheyden (kilpailun) vaikutusta. Naaras-tiheyden alentaminen poikasten hoitoaikana paransi emojen vieroitusmenestystä, ja sama vaikutus oli ravinnon lisäyksellä. Lack'in hypoteesin mukaan emojen kyky hankkia ravintoa on tärkein poikasten lukumäärää säätelevä tekijä. Tätä teoriaa tutkittiin manipuloimalla samanaikaisesti sekä emojen poikuekoko että ravintoa. Tulokset tukivat Lack'in teoriaa: kun lisäravintoa oli jatkuvasti saatavilla, emot kykenivät vieroittamaan menestyk-

sekkäästi alkuperäistä suuremman määrän poikasia. Lisääntyvien naaraiden tiheyden alentaminen kasvatti naaraiden liikkuma-aloja, mutta ei kuitenkaan vaikuttanut poikasten laatuun.

Lisääntyvät metsämyyränaaraat ovat territoriaalisia, eli ne puolustavat sitä osaa liikkuma-alastaan, joka ei mene päällekkäin muiden lisääntyvien naaraiden kanssa. Naaraiden territoriaalisuus oli kuitenkin selvästi riippuvainen lisääntymissyklin vaiheesta. Lisääntymissyklin edetessä kohti poikasten imetysvaihetta naaraiden aggressiivisuus kasvoi ja niiden liikkuma-alojen koko pieneni. Imetysvaiheessa emojen territorioiden koko vaikutti olevan suurimmillaan, mutta liikkuma-alojen koko pienimmillään. Ravintorikkaassa ympäristössä naaraiden liikkuma-alat olivat pienemmät, mutta liikkuma-alan koko poikasten hoitoaikana ei riippunut poikueen koosta. Myös lisääntymistiheys vaikutti tilankäyttöön: tiheimmässä populaatiossa emojen liikkuma-alat olivat pienemmät. Tämä tilankäytön joustavuus heijastelee territorion puolustamisesta aiheutuvien hyötyjen ja haittojen välistä allokointiongelmia.

Sekä laboratorioskokeiden että ulkotarhoissa tehtyjen kokeiden perusteella naaraspoikasen korkea syntymäpaino (ennemmin kuin vieroituspaino) ennustaa varhaisempaa ensimmäisen lisääntymisen ikää. Koska metsämyyräpopulaation tiheys kasvaa usein lisääntymiskauden edetessä, aikaisempi lisääntyminen saattaisi oikeuttaa rikkaampaan resurssiympäristöön. Emon lisääntymismenestyksen kannalta siis sekä suuri poikuekoko että poikasen koko on edullinen. Näiden piirteiden välillä havaittiin kuitenkin negatiivinen vuorovaikutussuhde: suuremmissa poikueissa poikaset olivat pienempiä. Molemmissa ominaisuuksissa mitattu heritabiliteetti (periytyvyys) oli myös korkea verrattuna yleensä havaittuihin kelpoisuuden kanssa korreloivien piirteiden heritabiliteettiarvoihin. Mahdollinen syy siihen, että valinnan myötä poikuekoossa ja poikasen koossa havaittava muuntelu ei ole hävinnyt, on piirteiden välillä löytynyt negatiivinen geneettinen vuorovaikutussuhde.

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ORIGINAL PAPERS

I

**Food limitation on reproductive success: an experiment in the bank vole
*Clethrionomys glareolus***

By

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FOOD LIMITATION ON REPRODUCTIVE SUCCESS: AN EXPERIMENT IN THE BANK VOLE *Clethrionomys glareolus*

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SUMMARY

Food limitation has been suggested as one of the most important factors affecting life-history evolution in terrestrial vertebrates. However, this inference is mainly based on evidence from birds, and reproductive trade-offs may differ among groups with different forms of parental care. To study whether the costs of enlarged litter (decreased mass of offspring) would appear when food is not limiting, we performed outdoor enclosure experiments in which we manipulated simultaneously the litter size (control vs. +2 pups) and food availability (control vs. food supplemented) of bank vole *Clethrionomys glareolus* females. The weaning success of females increased significantly in response to supplementary food. When females were provided with extra food, there were no differences in body masses of weanlings between control and enlarged litters. Further, food supplemented females grew to larger size during nursing than unsupplemented females. Our experiment suggests that energetic requirements during nursing constrain the number of offspring which can be successfully raised in a particular breeding attempt. The results also indicate that unlimiting food resources may increase future reproductive potential of females because they can use more energy for somatic growth.

INTRODUCTION

Lack's (1947) hypothesis, that clutch size in altricial birds is ultimately adjusted to the feeding capacity of the parents, has been tested in numerous studies (reviewed in Murphy & Haukioja 1986 and VanderWerf 1992). Although Lack's original argument has received some conflicting evidence, and has been refined over the years (e.g. Högstedt 1980, Boyce & Perrins 1987, Nur 1988), limiting food resources have been widely accepted as one of the most important factors affecting life history evolution in birds (reviewed in Martin 1987 and Boutin 1990). Lack (1948) urged researchers to test his hypothesis also in free-ranging mammals, but such experiments have been few. Mammals differ from birds in the form of parental care, and hence the reproductive trade-offs may differ between the groups.

The general result from brood size manipulations performed in birds (reviewed in Lindén & Møller 1989 and Roff 1992) and from an experiment in mammals (Mappes et al. 1995) is that brood enlargements do not increase the

number of high quality offspring parents can raise to independence. By supplementing food exclusively during the nestling period, it would be possible to study whether this is due to limited feeding capacity of parents (i.e. mother's ability to provide sufficient food for pups). This kind of evidence is surprisingly scarce, but in general the results demonstrate improved reproductive success (e.g. fledging success, offspring number and/or mass) of food supplemented parents compared to control nests (Arcese & Smith 1988, Simons & Martin 1990, Richner 1992, Wiehn & Korpimäki 1997, Siikamäki 1998).

The relationship between food resources and reproduction has also been widely studied in mammals (reviewed in Boutin 1990, O'Donoghue & Krebs 1992, Doonan & Slade 1995, Wauters & Lens 1995). However, these studies have mostly emphasised the effects of food addition at the population level. Duquette and Millar (1995) examined the influence of food addition on reproduction of individual tropical mouse *Peromyscus mexicanus* females. They found that food supplemented females had better weaning success than unsupplemented ones, whereas weanling mass was not affected by extra food. This experiment was conducted in unfenced grids and food was provided throughout the reproductive phase. However, there are no field experiments on mammals where the effects of food limitation on reproductive trade-offs of individual females have been studied while simultaneously controlling confounding factors (e.g. changes in densities of adult individuals due to immigration, emigration or increased reproduction, individual state, predation).

We used the bank vole as a study species to examine whether female reproductive success (size, number and proportion of weaned offspring per litter) is food limited. Further, we examined if food availability during nursing affects the body size and future breeding performance of the females. To do this, brood size (control vs. + 2 pups) and female food resources (control vs. supplemented) were simultaneously manipulated in large outdoor enclosures during nursing.

METHODS

Study site and study species

The study was conducted at Konnevesi, central Finland (62°37'N, 26°20'E) using eight 0.25 ha outdoor enclosures situated in an old field. Two separate replicates were carried out: the first in June - July and the second in July - August 1997. To monitor individuals, 25 multiple-capture live traps were distributed in each enclosure in a 5 x 5 array with 10 meters between the trap stations. Each trap was covered by a galvanised sheet metal chimney which served as a rainproof place for feeders (see below). Fences prevented the access of small mustelid predators to the enclosures and ensured zero emigration and immigration. For a more detailed description of the habitat and design of the enclosures see Koskela et al. (1997). The bank vole is well suited for the study, because it does not recognise it's pups from foreign ones (Mappes et al. 1995). In our study area the range of litter size of bank vole is from one to ten (usually 4-8), and breeding season lasts from late April to September (T. Mappes and R.

Koskela, unpublished data). The bank voles used in the study were wild-caught from nearby forests in May-June. All females had given birth at least once before the experiment. In the first replicate all females were over-wintered, in the second replicate some of the females were year-born.

Study design

To get enough pregnant bank vole females for the study ($n = 64$), females were paired at the same time both in the enclosures and in the laboratory. The females that were paired in enclosures were removed to the laboratory before parturitions. Females were inspected twice a day, and those giving birth within two days were chosen for the experiment. Right after pups were found, they were counted, weighed, marked and their sex was determined (by the length of anogenital distance). The proportion of females mated in the enclosures ($n = 41$) or in the laboratory ($n = 23$) were assigned equally to different treatment groups (Food treatment: $\chi^2 = 0.61$, $p > 0.4$, Litter-treatment: $\chi^2 = 0.07$, $p > 0.7$). Furthermore, the body mass, size (measured as width of head) or initial litter size did not differ between females mated in enclosure or laboratory (Two-sample t-tests, $p > 0.2$ for all the three variables).

The uterine environment and mother's quality may significantly influence the behaviour and life-history of individuals (reviewed in vom Saal 1981 and Clark & Calef 1995), and so cross-fosterings were performed to randomise prenatal maternal effects on performance of pups. Litters were manipulated and cross-fosterings performed within two days of parturition. All pups in a litter were changed in the cross-fosterings. According to a previous experiment, the growth and survival of bank vole pups do not differ between the female's own pups and foreign pups (Mappes et al. 1995).

To manipulate food resources during nursing we had two treatments: supplemented, where food was provided *ad libitum* at every trap station, and control, where no supplemental food was available in the enclosures. The bank voles normal food in the enclosure habitat consists mainly of forbs and seeds (Larsson & Hansson 1977). Supplemental food was laboratory rodent chow (Labfor R36) and it was provided in wiremesh feeders which prevented food hoarding by the voles. Feeders were set out at the same time when females and pups were released to the enclosures and removed 20 days later. In litter size manipulations, we assigned litters of each original size randomly to two treatment groups: enlarged litters, where two pups were added, and control litters, where the original litter size was not changed. So, as a result of the manipulations (in both replicates) we had four food supplemented enclosures and four unsupplemented enclosures with four females in each, two females nursing a control litter and two an enlarged litter (total $n = 64$). The assignment of enclosures into food and control treatments was reversed between replicates. At the beginning of the experiment there were no significant differences in initial litter sizes, body masses or sizes of mothers between treatments or enclosures (treatments: Two-way ANOVAs, p for all > 0.2 , enclosures: Kruskal-Wallis one-way ANOVAs, p for all > 0.7).

After the manipulations were performed in the laboratory, females with litters were transferred in breeding cages to enclosures. Cages were placed near the corners of the enclosures (in rainproof covers) 7.5 meters away from the fences. Cages were left open so that the mothers could move pups into the enclosures. This method has been successful in previous studies (Mappes et al. 1995). In natural populations small mammal females with postpartum oestrus are usually pregnant at the same time when lactating (e.g. Bronson 1989). In the first replicate, pregnancy was made possible by introducing three mature males into each enclosure after releasing females. In the second replicate males were not released to the enclosures, instead, all females were given the opportunity to mate in the laboratory. Mating procedure was different in the second replicate to enable the estimation of the amount of extra food eaten by nursing females (P. Jonsson, T. Hartikainen, E. Koskela & T. Mappes, unpublished). There was no difference in the proportion of subsequent pregnancy in mothers between replicates (79 % and 80 % of females pregnant in 1st and 2nd replicate, respectively, $\chi^2 = 0.01$, $p = 0.940$).

Before expected parturitions of subsequent litters (ca. 20 days after possible matings), females were removed from enclosures, measured and the number of embryos was determined. Successfully weaned offspring (from manipulated litters) were captured and taken to the laboratory at 30 days age and they were individually weighed (weaning mass) and measured (width of head).

Data analysis

As parameters of reproductive success we used the proportion (arcsine squareroot transformed) and number of weaned young per litter, and the size (body mass and head width) of weaned offspring. The effect of manipulations on growth of mothers was examined by measuring the head width of females before and after the study. Head width was more appropriate for this purpose than weight because it is not directly affected by pregnancy. Head width also correlates well with structural size (T. Mappes & E. Koskela unpublished).

The possible effects (or interactions) of replicate and enclosure on dependent variables were examined using analyses of variance. If any tendency for differences between groups to occur were found ($p < 0.1$), the factor was included in the analyses with dependent variables. To avoid pseudoreplication, the offspring size and mass at weaning were analysed using ANOVA models where individual offspring of the foster female were nested within food and litter manipulations (see Zar 1996). A total of 192 weanlings were found and the number per individual mother ranged from 0 to 9. To proceed the analyses of weanling mass and size successfully (no redundancies in design matrix, SPSS Inc. 1992), the variation in offspring number per female had to be decreased to maximum of six young. This was done by randomly removing data on required number of offspring (a total of 12 young) from eight mothers originally having more than six weanlings. The procedure makes it possible to include the within-foster female variation to the analyses. The following abbreviations are used when referring to the four treatment groups: 1) no food added, control litters

'CC', 2) no food added, enlarged litters 'CE', 3) extra food, control litters 'FC', 4) extra food, enlarged litters 'FE'. Only the mothers that were alive throughout the study ($n = 54$) were included in the analyses. When the assumptions of parametric tests were not met, non-parametric tests were used. All the tests were two-tailed. The level of statistical significance was set to $\alpha = 0.05$, and probability values between 0.05 and 0.1 were considered only as a tendency for finding a real effect.

RESULTS

Weaning success and number of offspring

The proportion of weaned offspring per litter (weaning success) increased significantly in response to supplementary feeding, whereas there was no difference between litter manipulation groups (ANOVA, Replicate: $F_{(1,49)} = 4.84$, $p = 0.033$, Food: $F_{(1,49)} = 5.24$, $p = 0.026$, Litter: $F_{(1,49)} = 1.11$, $p = 0.296$, Food by Litter: $F_{(1,49)} = 0.10$, $p = 0.758$; Fig. 1). After litter size manipulations, the number of offspring was higher in enlarged litters than in control litters, but there was no difference between food treatments (ANOVA, Food: $F_{(1,60)} = 0.05$, $p = 0.821$, Litter: $F_{(1,60)} = 43.65$, $p < 0.001$, Food by Litter: $F_{(1,60)} = 0.47$, $p = 0.497$; Fig. 2). However, there was a slight tendency for number of young at weaning to be greater with supplemental food, whereas there was no difference in litter size between litter manipulation groups (ANOVA, Food: $F_{(1,50)} = 3.24$, $p = 0.078$, Litter: $F_{(1,50)} = 0.33$, $p = 0.567$, Food by Litter: $F_{(1,50)} = 0.26$, $p = 0.612$; Fig. 2).

Body mass and size of offspring

Food supplementation increased both the body mass and size of weaned offspring (Table 1, Figs. 3a,b). Without extra food, the mass and size of offspring in enlarged litters were lower as compared to the control litters. Furthermore, a significant litter by food manipulation interaction indicated that when the females were food supplemented, there were no differences in body masses of weanlings between control and enlarged litters (Table 1, Figs. 3a,b).

Characteristics of mothers and subsequent breeding

A total of ten mothers died (disappeared) during the experiment without any obvious difference between four treatment groups (CC 4, CE 2, FC 1, FE 3). In general, the size of the mothers increased during the experiment. When provided with supplemental food, mothers grew significantly more than the unsupplemented ones, whereas litter manipulation did not have any significant effect on growth (ANOVA, Replicate: $F_{(1,46)} = 16.83$, $p < 0.001$, Food: $F_{(1,46)} = 5.11$, $p = 0.029$, Litter: $F_{(1,46)} = 0.25$, $p = 0.622$, Food by Litter: $F_{(1,46)} = 0.05$, $p = 0.821$; Fig. 4).

The proportion of females producing subsequent litters in the four treatment groups was as follows: CC 58 % ($n = 12$), CE 86 % (14), FC 80 % (15), FE 92 % (13). The probability of subsequent breeding was analysed using a Logit-model with breeding as a dependent variable, and food and litter

manipulations as explaining factors. In the analyses food did not affect subsequent breeding, but there was a tendency, although not significant, for females nursing enlarged litters to be more likely to breed (Logit-model, Food: $\chi^2 = 1.76$, $df = 1$, $p = 0.185$, Litter: $\chi^2 = 3.36$, $df = 1$, $p = 0.067$, Food by Litter: $\chi^2 = 0.05$, $df = 1$, $p = 0.820$). Subsequent litter size tended to be larger in response to supplementary feeding, whereas litter enlargements did not affect subsequent litter sizes (mean \pm s.e., CC: 5.3 ± 0.6 , CE: 6.1 ± 0.4 , FC: 6.6 ± 0.3 , FE: 6.2 ± 0.2 , ANOVA, Food: $F_{(1,36)} = 3.50$, $p = 0.069$, Litter: $F_{(1,36)} = 0.25$, $p = 0.622$, Food by Litter: $F_{(1,36)} = 2.62$, $p = 0.114$).

DISCUSSION

According to our results, weaning success of bank vole females is limited by food availability. When females were provided with extra food, they weaned larger (body mass and size) offspring than control females. Enlarging the litter size decreased the body mass and size of weaned offspring (as in Mappes et al. 1995). However, when food was supplemented, offspring body mass was unaffected by litter enlargement. This indicates that the nursing effort of mothers (i.e. the amount of milk for pups) was limited by food availability. Female body size was also affected by extra food: with supplemental food females grew bigger during nursing. The results also indicate that food availability may increase the subsequent litter size of females but not the probability of subsequent breeding. However, these latter non-significant results may be caused by inadequate sample sizes and should be taken as questions for further studies.

Food supplemented females had better weaning success and tended to wean more offspring than unsupplemented females. This can be considered as an obvious evidence for food limiting reproductive success of bank vole females. The size at fledging has been found to explain future survival or probability of breeding in birds (e.g. Perrins 1965, Gustafsson & Sutherland 1988) but similar evidence for size at weaning is scarce in mammals. Most of the studies demonstrating benefits of large size are correlative and have not controlled for maternal effects arising from mother quality or number of offspring (e.g. Myers & Master 1983, Dobson & Michener 1995). However, higher mass at weaning of autumn-born bank vole females has been found to correlate with over winter survival probability (Koskela 1988). Further, the probability of bank vole females to start reproducing during the summer of birth increases with higher body mass at weaning (Mappes et al. 1995). Consequently, larger size of weanlings may indicate better quality and suggests higher reproductive success for food supplemented mothers.

Positive effect of food on size and/or growth of adults has been found in most studies on birds and mammals (review in Boutin 1990, Garcia et al. 1993, Wiehn & Korpimäki 1997). In the current study, females with extra food grew larger by the end of the experiment. Large size may reflect better condition and better reproductive potential in future, e.g. head width of bank vole females correlated positively, although not significantly, with litter size ($r_s = 0.22$, $n = 64$, $p = 0.081$). Furthermore, in the present study food supplemented females

tended to have larger subsequent litters than control females. This would suggest that the litter size in bank voles is affected by physiological condition and/or future breeding environment of females and their offspring.

The proportion of weaned offspring per litter was higher in the supplemental food treatment than in control treatment, suggesting that survival of pups is limited by food availability. Because extra food was removed before pups were in a trappable age, we suggest that the effect of food on offspring was mediated through better nursing ability of mothers. We propose three possible mechanisms for different mortality of pups between food treatments: 1) dying from undernourishment or starvation, 2) dying from detrimental effects of adults during partial independence from mother (Boonstra 1978) or 3) killing of pups in their nest by neighbouring females (infanticide; Ylönen et al. 1997). We cannot differentiate between these factors, because we were unable to monitor the survival of pups before trappable age. However, in the present study the home ranges of females were smaller when food was supplemented (P. Jonsson, T. Hartikainen, E. Koskela & T. Mappes, unpublished). This might indicate decreased disturbance caused by adult females towards juveniles and/or cause smaller risk of infanticide. We conclude that the weaning success of mothers increases with supplemental food when density-dependent effects are controlled. However, the complete mechanism is unclear.

To conclude, our study suggests that energy requirements of lactating females is an important constraint on the number of offspring which can be successfully raised, in agreement by Lack's (1948) argument. Availability of food resources during nursing may also affect individuals future reproductive success because females can use more energy for somatic growth. Whether the reproduction is food limited also in bank voles' more common habitats in old deciduous or spruce forests (according to Myllymäki 1977) remains open. Also, in enclosed populations the normal dispersal of voles is not possible, which may confound the results. Obviously more experimental studies are needed for generalising the results for mammals.

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Table 1. Body mass and size (head width) of weanlings in different treatments. (Food = food manipulation, Litter = litter manipulation. ANOVA models used where individual offspring of the foster female (random effect) were nested within treatments (fixed effects). See also figures 3a, b).

	d.f.	MS	F	p
Body mass				
Food	1	92.96	44.47	< 0.001
Litter	1	14.77	7.07	0.015
Food * Litter	1	10.94	5.23	0.033
Foster female	20	2.09	0.79	0.719
Error	156	2.64		
Total	179			
Head width				
Food	1	3.49	33.41	< 0.001
Litter	1	0.62	5.91	0.025
Food * Litter	1	0.09	0.9	0.353
Foster female	20	0.10	0.83	0.671
Error	151	0.13		
Total	174			

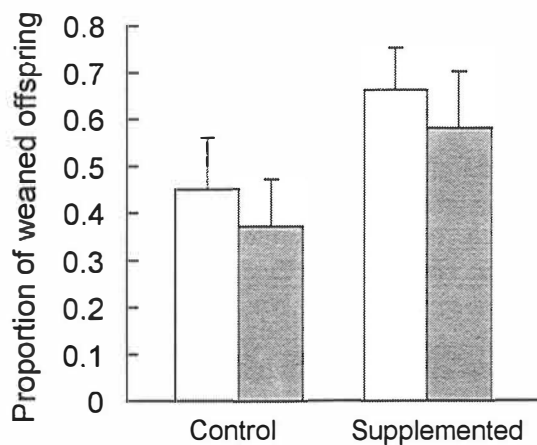


Fig. 1. Proportion of weaned offspring per litter (weaning success) in food supplemented (Supplemented) and control (Control) females. Control litters = white bars, enlarged litters = black bars. Bars show the mean \pm s.e (untransformed values).

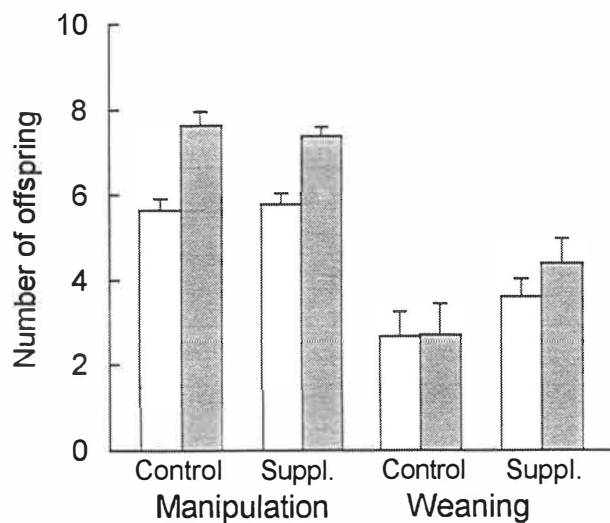
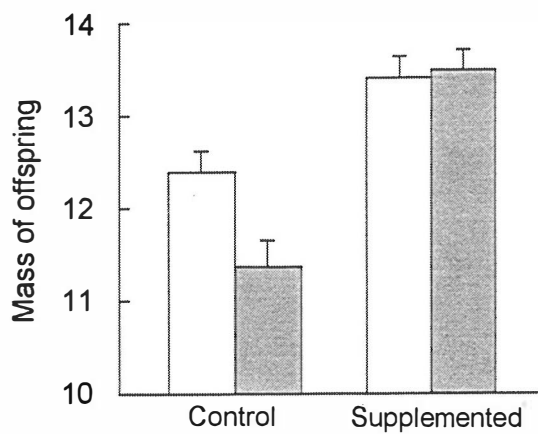


Fig. 2. Number of offspring per female after manipulation and at weaning in different treatments. Control = unsupplemented females, Supplemented = food supplemented females, Control litters = white bars, Enlarged litters = black bars.

A)



B)

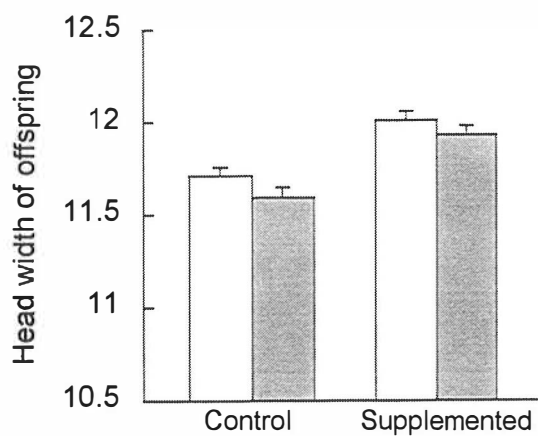


Fig. 3. Characteristics of offspring at 30 days in food supplemented (Supplemented) and control (Control) treatment. (a) Body mass (in grams). (b) Body size (head width, in mm). Control litters = white bars, enlarged litters = black bars. Bars show the mean \pm s.e. For statistics see the Results.

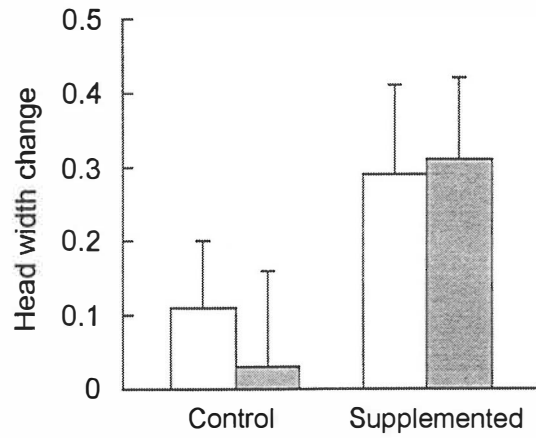


Fig. 4. The growth of females during the experiment measured as head width change (in mm) in food supplemented (Supplemented) and control (Control) treatment. Control litters = white bars, enlarged litters = black bars. Bars show the mean \pm s.e. For statistics see the Results.

II

**Experimental manipulation of breeding density and litter size: effects on
reproductive success in the bank vole**

By

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EXPERIMENTAL MANIPULATION OF BREEDING DENSITY AND LITTER SIZE: EFFECTS ON REPRODUCTIVE SUCCESS IN THE BANK VOLE

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SUMMARY

1. Reproductive success of individual females may be determined by density-dependent effects, especially in species where territory provides the resources for a reproducing female and territory size is inversely density-dependent.
2. We manipulated simultaneously the reproductive effort (litter size manipulation: ± 0 and $+2$ pups) and breeding density (low and high) of nursing female bank voles *Clethrionomys glareolus* in outdoor enclosures. We studied whether the reproductive success (number and quality of offspring) of individual females is density-dependent, and whether females can compensate for increased reproductive effort when not limited by saturated breeding density.
3. The females nursing their young in the low density weaned significantly more offspring than females in the high density, independent of litter manipulation.
4. Litter enlargements did not increase the number of weanlings per female, but offspring from enlarged litters had lower mass than control litters.
5. In the reduced density females increased the size of their home range, but litter manipulation had no significant effect on spacing behaviour of females. Increased home range size did not result in heavier weanlings.
6. Mother's failure to successfully wean any offspring was more common in the high density treatment, whereas litter manipulation or mother's weight did not affect weaning success.
9. We conclude that reproductive success of bank vole females is negatively density-dependent in terms of number but not in the quality of weanlings.
10. The nursing effort of females (i.e. the ability to provide enough food for pups) seems not to be limited by density-dependent factors.

INTRODUCTION

Reproductive success (i.e. number and quality of offspring produced) of a female is determined by trade-offs between different life-history traits, constraints and several ecological factors. Of the ecological determinants, intraspecific competition and density-dependent effects on reproduction have received much attention, particularly in birds (e.g. Perrins 1965, Lundberg et al. 1981, Nilsson 1987, Cooch et al. 1989) and also to some extent in mammals (e.g.

Clutton-Brock, Guinness & Albon 1982, Morris 1989, Wauters & Lens 1995). In general, breeding success of females seems to be negatively correlated with density and competition between individuals, possibly due to limited food resources. This has further been experimentally studied both in birds (e.g. Alatalo & Lundberg 1984, Török & Tóth 1988) and mammals (Ostfeld, Canham & Pugh 1993, Ostfeld & Canham 1995). However, in mammals studies have concentrated on reproductive success at the population level (mean number of recruits to the population), and density-dependent effects on reproductive success of individual females have not been experimentally studied.

Increasing density may influence the average reproductive output in a population by affecting the reproductive success of all females (Lack 1954), or by forcing more females to breed in poor-quality breeding sites where reproductive success is lower (Andrewartha & Birch 1954). Density-dependence in brood size in heterogeneous environments has been observed both in birds (Dhondt, Kempenaers & Adriansen 1992) and in small mammals (Morris 1992). However, density-dependent changes in reproductive success may also occur regardless of environmental heterogeneity. Increasing the density of reproducing females may reduce the size of territories in small mammals (e.g. Mazurkiewicz 1971, Viitala 1977, T. Mappes and E. Koskela unpublished data), but there is no evidence of that in birds (according to Dhondt et al. 1992). Reduced territory size may consequently decrease the amount of food resources and/or the number of secure nest sites within each territory. Territory quality may affect reproduction of individual females by proximately constraining their reproductive effort (e.g. females have limited amount of food) or alternatively, by adaptive adjustment of their reproductive effort according to the quality of territory (individual optimization hypothesis, Perrins & Moss 1975, Morris 1985, Pettifor, Perrins & McCleery 1988). Until now most of the manipulations of breeding density of female birds (e.g. Tompa 1967, Alatalo & Lundberg 1984, Virolainen 1984, Török & Tóth 1988) have been conducted by offering nest-boxes in different densities before females have started laying eggs. This study design lacks the ability of randomizing the females into different treatments. Further, to be able to control for the possible adjustment of brood size to density, the density manipulation should be performed after females have given birth. Territorially breeding species in which the territory provides all resources for a breeding female, offer a good possibility to study density-dependence of reproductive success by manipulating density of breeding females. This is not possible in many birds, in which foraging trips outside of the territory are frequent and the estimation of territory quality is difficult. In contrast, in many territorially breeding small mammals, home range provides all resources for a breeding female, and density manipulations can be carried out at every stage of the reproductive cycle.

In our study species, the bank vole *Clethrionomys glareolus* Schreber, breeding females are territorial (e.g. Bujalska 1973, Koskela, Mappes & Ylönen 1997) and home range sizes of nursing females have been found to be negatively correlated with density (T. Mappes and E. Koskela, unpublished data). We recently studied reproductive effort and reproductive costs in a litter size manipulation experiment in the bank vole (Mappes, Koskela & Ylönen

1995). In that study, bank vole females did not seem to compensate increased nursing costs with increased parental effort. In other words, they did not trade their own condition against the quality of offspring or enlarged the size of their home range during nursing. We proposed two different explanations for this (Mappes et al. 1995): 1) Females attempted to maximise their survival probability during the present breeding event or to the next breeding attempt, which may maximize their lifetime reproductive success (Williams 1966) or 2) Density-dependent factors in saturated breeding density limited the current nursing effort (i.e. the ability to provide sufficient food for pups).

We used a novel experimental approach to study the effects of breeding density and litter size on reproductive success in female bank voles. In particular, we studied the density-dependent effects on reproductive success in female small mammals during nursing, at the time when the energy needs are greatest (Kaczmarski 1966, Gittleman & Thompson 1988). By manipulating the litter size and breeding density simultaneously, we created a situation where females had the possibility to compensate for increased nursing costs by enlarging their territories to obtain sufficient amounts of resources for the current breeding attempt. Because especially in mammals the prenatal environment (e.g. intrauterine position) and mother's quality may have significant influence on behavior and life history strategies of individuals (reviewed in vom Saal 1981 and Clark & Galef 1995), cross-fosterings were performed to randomize the influence of prenatal maternal effects on the performance of pups.

MATERIALS AND METHODS

Study site and animals

The study was conducted at Konnevesi, central Finland (62°37'N, 26°20'E) in 0.25 ha outdoor enclosures. Because of a limited number of enclosures in use, four separate runs of the experiment were carried out: 1st in May-June (five enclosures in use), 2nd in June-July (three enclosures), 3rd in July-August (four enclosures) and 4th in August-September (two enclosures). For monitoring the individual voles, 25 multiple-capture live traps were distributed in each enclosure in a 5 x 5 array with 10 meters between the trap stations. For a detailed description of the habitat and design of the enclosures see Koskela et al. (1997). All female voles used in the study were wild-caught and had overwintered, except for females in the second run which were young-of-the-year and originated from a laboratory colony. The normal range of litter size for bank vole females in our study area is from one to ten (usually 4-8, T. Mappes and E. Koskela unpublished data). The males were wild-caught and showed scrotal testes as a sign of their maturity.

Study design

At the start of the experiment six (individually marked) females were randomly assigned to each enclosure and released simultaneously in the middle of each

enclosure. This density is comparable to the highest breeding densities observed in earlier studies (Bujalska 1970, Ylönen, Kojola & Viitala 1988). There were no differences in the size of females (body weight and width of head) in different enclosures at the beginning of the study (one-way ANOVA, $p > 0.9$ for both variables). After a seven days habituation period three mature, randomly chosen males were introduced into each enclosure. The spacing dynamics of individuals were monitored two times during the experiment: 1) when females were in the late pregnancy of their first litters and 2) after litter size and density manipulations when females were nursing their young. During trapping periods traps were checked 10 times, twice a day (morning and evening) for five days. At each capture, vole identity, sex, trap location, weight and reproductive status were recorded.

After the first trapping period (late pregnancy), 17 days after releasing males, all females were removed from enclosures and housed in standard breeding cages in the laboratory until they gave birth. The breeding of females within enclosures were in close synchrony: all gave birth within 1-4 days. Mothers were inspected twice a day for parturition. Right after pups were found, they were counted, weighed and their sex was determined (by the length of the anogenital distance). Litters were manipulated and cross-fosterings performed within two days from the birth. In cross-fosterings all pups in a litter were changed (no littermates in the same litter) and when possible, the sex ratio of litters was not changed. In our previous experiment, the growth or survival of pups did not differ between the female's own pups and foreign pups (Mappes et al. 1995). Nursing density was manipulated in two treatments: reduced, where two randomly selected females from the same enclosure were released to their original enclosure with pups, and control, where the density of females was not changed from the original six. In litter size manipulations we assigned litters of each original size randomly to two treatment groups: enlarged litters, 'E', where two pups were added and control litters, 'C', where the original litter size was not changed. The pups for enlarged litters originated from mothers that were not released back to the enclosures (low density treatment). So, as a result of the manipulations we had high density enclosures with six females in each, three nursing control and three enlarged litters, and low density enclosures with two females in each, one female nursing a control and one an enlarged litter. The original litter size did not differ among the density treatments or litter manipulation groups (Three-way ANOVA, Run: $F_{(3,41)} = 2.51$, $p = 0.072$; Density: $F_{(1,41)} = 1.54$, $p = 0.222$; Litter: $F_{(1,41)} = 0.37$, $p = 0.544$; Density * Litter: $F_{(1,41)} = 0.02$, $p = 0.879$). After performing manipulations within the enclosures, females and their litters (pups individually marked) were returned in breeding cages to enclosures and placed in the activity center which should lie very near to their nests (Mironov 1990). Cages were left open so the mothers could carry pups back to the nests. This method has operated well in our previous studies (Mappes et al. 1995).

When offspring were 30 days old, they were captured and taken to the laboratory where they were individually weighed (= weaning weight). Before the possible birth of second litters, females were removed from enclosures to the laboratory to determine the characteristics of subsequent breeding (litter

size, birth weight of pups, postpartum weight of mothers). During these two last trapping sessions trapping was continued until all individuals were caught (in the case of mothers) or new individuals were not found (weanlings).

Home range sizes and activity centers were estimated for individuals separately for two trapping periods. Home ranges were calculated using 90% mononuclear probability polygon centred on arithmetic mean (90% MPP) (Kenward 1987). Home range size was not correlated with the number of captures (before manipulations: $r_s = 0.075$, $p > 0.6$, after manipulations $r_s = -0.025$, $p > 0.9$). The activity center was calculated as the arithmetic mean point of each individual's capture co-ordinates.

Data analysis

As the four runs of the experiment were carried out in different seasons it is necessary to take into account possible effects of changing environment on the results. Our experiment did not aim to study seasonal effects, as for that the sample sizes would have been too low. However, in the analyses of breeding success of females study run was included in the models as a separate factor. In other analysis the effect of run was studied first and if found significant ($p < 0.1$) it was included in the analysis. Within each run, the possible effect of enclosure on dependent variables (home range size, litter size, birth weight, weaning weight) was studied using either one-way ANOVA or Kruskal-Wallis one-way ANOVA (depending on whether the assumptions of parametric test were met). We did not find any significant effect of enclosure on any trait (p for all > 0.1), and hence, the enclosure was not used as a separate factor in the following statistical analyses. A total of 141 weanlings were caught of which 112 were weighed at birth and 139 at weaning. In the analyses of offspring weight (at birth and at weaning) between treatments the mean values of litters of foster mothers were used. Possible factors behind total losses of litters (weaning success) were studied using Logistic regression and Log-linear models. For correlation analyses Spearman rank correlations were used. All the tests are two-tailed. The statistical analyses were performed by using SPSS for Windows (SPSS Inc. 1992).

RESULTS

Number and quality of weanlings and weaning success

After litter size manipulations in the laboratory, the number of offspring differed significantly between litter manipulation groups but not between density treatments (Fig. 1, Table 1). However, the number of young weaned per female was greater in the low density treatment, but there was no significant difference between litter manipulation groups or interaction between litter and density treatments (Fig. 1, Table 1). If the analysis is performed only with females that weaned at least one young (see below), the size of litters at weaning were not significantly different between the litter manipulation groups regardless of nursing density (Control density: C litters 4.1 ± 0.5 , E litters $5.2 \pm$

1.1, Reduced density: C litters 4.5 ± 0.7 , E litters 5.9 ± 0.7 , Three-way ANOVA, Run: $F_{(3,22)} = 0.74$, $p = 0.538$; Density: $F_{(1,22)} = 0.17$, $p = 0.686$; Litter manipulation: $F_{(1,22)} = 1.68$, $p = 0.208$; Density * Litter: $F_{(1,22)} = 0.08$, $p = 0.775$).

Nineteen out of 48 females released in the enclosures with their litters failed to wean successfully any young to the age of 30 days (Table 2). Factors affecting weaning success were studied using Logit-model with weaning success as a dependent variable and density and litter manipulations as explanatory factors (Table 3). All the models which included density-factor fitted to the data significantly ($p > 0.05$). In further analyses density of nursing females explained significantly the weaning success of mothers ($G = 6.889$, $df = 1$, $p = 0.009$), so that breeding failures were more common in high as compared to low density. The effects of litter manipulation or the interaction term between the two treatments were not significant (Litter: $G = 0.908$, $df = 1$, $p = 0.341$, Interaction: $G = 0.036$, $df = 1$, $p = 0.849$). Weaning success was not affected by the number of pups the mother was nursing after manipulation (Logistic regression, $G = 1.58$, $n = 48$, $df = 1$, $p = 0.691$) or mother's postpartum weight (Logistic regression, $G = 0.18$, $n = 44$, $df = 1$, $p = 0.671$). Neither did the number of disappeared offspring correlate with mother's initial litter size (Pooled data from both density manipulation groups; for C litters: $r_s = 0.162$, $n = 24$, $p = 0.450$, for E litters, $r_s = 0.110$, $n = 24$, $p = 0.610$).

At manipulation, the initial mean weight of pups did not differ among the treatments (Three-way ANOVA, Run: $F_{(3,36)} = 0.35$, $p = 0.791$, Density: $F_{(1,36)} = 0.17$, $p = 0.681$, Litter: $F_{(1,36)} = 0.60$, $p = 0.443$, Density * Litter: $F_{(1,36)} = 0.17$, $p = 0.686$). At weaning, the weight of offspring tended to be lower in enlarged litters compared with the control group but the effect of density was not significant (Three-way ANOVA, Run: $F_{(3,22)} = 0.63$, $p = 0.606$, Litter: $F_{(1,22)} = 3.45$, $p = 0.077$, Density: $F_{(1,22)} = 0.00$, $p = 0.968$, Litter * Density: $F_{(1,22)} = 0.00$, $p = 0.963$, Fig 2.). However, if we control for variation in birth weight by introducing it to the model as a covariate, the effect of litter manipulation is significant (Run: $F_{(3,16)} = 3.04$, $p = 0.059$, Litter: $F_{(1,16)} = 4.76$, $p = 0.044$, Density: $F_{(1,16)} = 1.12$, $p = 0.305$, Covariate: $F_{(1,16)} = 5.15$, $p = 0.037$). Female home range size did not correlate significantly with the weight of weanlings ($r_s = -0.056$, $n = 28$, $p = 0.777$).

Spacing behavior and litter size

Before manipulations the home range size of females did not differ between treatments (Three-way ANOVA, Run: $F_{(3,37)} = 3.18$, $p = 0.035$, Density: $F_{(1,37)} = 0.53$, $p = 0.472$, Litter: $F_{(1,37)} = 0.03$, $p = 0.854$). Females enlarged the size of their home range in response to reduced density, but litter size manipulation had no effect on home range size (Run: $F_{(3,40)} = 3.32$, $p = 0.029$, Density: $F_{(1,40)} = 11.36$, $p = 0.002$, Litter: $F_{(1,40)} = 0.69$, $p = 0.410$, Density * Litter: $F_{(1,40)} = 0.04$, $p = 0.843$, Fig. 3). The body weight and head width of females at the start of the experiment correlated significantly with their initial litter size (Weight: $r_s = 0.492$, $n = 47$, $p < 0.001$, Head: $r_s = 0.534$, $n = 47$, $p < 0.001$). Home range size of females before manipulations did not correlate with initial litter size ($r_s = 0.010$, $n = 43$, $p = 0.951$).

Reproductive costs

Two females died after experimental manipulations, one in the enclosures and one in the laboratory before the birth of second litter. Both these females were from control density, one from a C litter and the other from an E litter. None of the eight females in the last run of the study had a subsequent litter, most likely because the breeding season was almost over. These females have not been included in the following analyses of subsequent breeding. Descriptive statistics for characteristics of subsequent breeding in different treatments are given in Table 4.

Subsequent breeding of mothers was studied by using logit-models with reproduction as the dependent variable. Litter and density manipulation groups and the weaning success of mothers (did mothers successfully wean any pups to trappable age) were used as explanatory factors in the model. According to low Z-values ($|z| < 0.5$) of parameter estimates from the saturated model, the weaning success-factor was excluded from further models. This was supported by the fact that, if analyzed separately, the success-factor did not affect the probability of subsequent breeding (Pearson's $\chi^2 = 0.17$, $p = 0.676$). All the models (including the constant effect) with litter and density manipulation as explanatory factors fitted the data significantly. In further analyses neither the effect of density manipulation ($\chi^2 = 1.709$, $df = 1$, $p = 0.191$) nor litter manipulation ($\chi^2 = 1.565$, $df = 1$, $p = 0.211$) on probability of second breeding were significant. The size of subsequent litters, mean birth weight of pups, or female weight change during the experiment (postpartum weight after subsequent litters minus initial weight) did not seem to differ between manipulation groups and were not affected by success in the previous breeding attempt (Table 5).

DISCUSSION

We used a novel approach to study the effects of breeding density and litter size on reproductive success of bank vole females. We further studied whether density-dependent factors, such as territory size and/or number of neighbouring females, limit the nursing effort (i.e. the ability to provide sufficient food for pups) of females. According to the results, density had clear effects on the reproductive success of females: mothers nursing their young in the low density weaned significantly more offspring than mothers in the high density. Litter enlargements did not increase the number of weanlings per female, but offspring from enlarged litters suffered from lower mass as compared to offspring from control litters. This was true independently of density treatment: even though the mothers increased the size of their home ranges when unlimited by saturated breeding density, it did not result in better quality (i.e. heavier) weanlings. This indicates that density-dependent factors were not limiting the nursing effort of bank vole mothers.

Density-dependent effects on reproductive success

Earlier breeding density manipulations studying the reproductive success of individual females have been conducted in birds, particularly in the pied (*Ficedula hypoleuca* Pall.) and collared flycatcher (*F. albicollis* Temm.) (Tompa 1967, Alatalo & Lundberg 1984, Virolainen 1984, Török & Tóth 1988). The general finding from these experiments is that the negative effects of density on breeding success of females are manifested in lower numbers and quality of fledglings, probably due to competition for food during the nestling period. In the present study, the reproductive success of mothers in terms of number of offspring weaned was strongly density-dependent. However, while density did not affect the weight of weanlings, litter enlargements seemed to have a negative effect on weanling weights as found also in other studies (Mappes et al. 1995, Koskela 1998, Koskela et al. 1998). There was no interaction between litter and density manipulations in the quality of weanlings. Hence, it seems that density-dependent factors do not limit the nursing effort of female bank voles. We recently carried out a food manipulation experiment in the same enclosures where the present study was conducted. The results suggest that the reproductive success of females in the enclosures is actually food limited: when supplied with extra-food, females decreased the sizes of their home ranges and weaned heavier offspring (Koskela et al. 1998, P. Jonsson, T. Hartikainen, E. Koskela and T. Mappes unpublished data).

The weaning success of females (probability to wean at least one young) was strongly density-dependent, independent of litter size or mother's weight. In the current study, the number of offspring weaned was determined when the pups were 30d old and most of young were probably independent of their mother (if the mother has given birth to a second litter). Thus, it is unclear whether most of the litter losses took place at very early stages of lactation, or whether postweaning mortality was important determinant of the pup survival. Infanticide (killing of foreign pups by conspecifics) has been observed in many small mammal species including the bank vole (Ylönen, Koskela & Mappes 1997) and it might be one cause of unsuccessful breedings. Infanticide occurs soon after birth and usually all the pups in the nest are lost. When density increases, pups may be more exposed to infanticide because the probability that an infanticidal individual encounters the nest is greater, as suggested in Mallory and Brooks (1978) (but see Boonstra 1980). Furthermore, the detrimental effect of adults, particularly adult females, on juvenile survival has been observed in many small mammal species, indicating that pup loss may also happen later in life (e.g. Boonstra 1978, Rodd & Boonstra 1988 and ref. therein, but see Ostfeld & Canham 1995). In this study, the greater mean number of weanlings per mother in the low density treatment did not result in lower weanling weight as compared to high density treatment. Also, because the litter sizes did not differ at weaning between density treatments (if only successful mothers were included), this suggest that most of the offspring mortality occurred at early age. However, we conclude that firm conclusions of the mechanisms causing lowered reproductive success in higher density can not

be drawn from the current data. It is possible that both infanticide and postweaning mortality are more frequent in high as compared to low density.

Reproductive costs

Mappes et al. (1995) proposed two different explanations for why mothers do not trade off their condition against the quality of pups. First, females can increase their survival probability during the present breeding event and/or to the next breeding attempt, which may maximize their lifetime reproductive success (Williams 1966). Second, intraspecific competition in saturated breeding density may limit the current breeding effort. The results from the present experiment do not support the latter hypothesis as there was no difference in the weight of offspring between the density treatments, and emphasize the importance to study reproductive costs as determinants of optimal reproductive effort in small mammals. However, earlier experimental studies in mammals have not found evidence of reproductive costs in terms of reduced fecundity or condition of mothers (Hare & Murie 1992, Mappes et al. 1995). Although our main aim in the present study was not to examine reproductive costs, the data gave us an opportunity to cautiously investigate the possible joint effects of density and litter manipulations on subsequent performance of females. We did not find any significant results showing differential survival or fecundity between females from different treatments. However, the used experimental design (brood enlargement) does not necessarily "force" females to increase their effort (e.g. Smith et al. 1988, Moreno et al. 1995, Tolonen & Korpimäki 1996) and therefore may fail to measure costs which do exist. Furthermore, considerable sample sizes are required for reliable statistical analyses of the probability of subsequent breeding (e.g. Graves 1991, Roff 1992). Pooling the data from our earlier study (Mappes et al. 1995) and from the control (high) density of the present study, gives the proportions of females producing subsequent litters 72% ($n = 25$) in the control litters and 52 % ($n = 27$) in enlarged litters ($\chi^2 = 2.23$, $df = 1$, $p = 0.136$, $\alpha = 0.05$, Effect size $w = 0.2$, Power of test = 0.30, from tables in Cohen 1988). With the present sample size, the power of test would be 0.80 (the desired power value proposed by Cohen 1988) only if the effect size would be 0.4, twice as high as it is now. In other words, with our sample size we would have an 80% probability of rejecting the null hypothesis only if the probability not to reproduce in experimental females would be twice as high as found.

Trade-off between number and quality of offspring

Is the quality of smaller individuals lower than that of the larger ones in terms of future survival and reproductive success? Size is usually considered to be positively correlated with fitness of an individual: larger individuals often enjoy a competitive advantage in reproduction or have faster growth rate and/or better survival (Roff 1992). However, there are surprisingly few data on subsequent performance (e.g. growth, survival, reproductive success) of different sized offspring in small mammals. Further, the studies conducted (e.g.

Fleming & Rauscher 1978, Myers & Master 1983, Kaufman & Kaufman 1987, Derrickson 1988, Solomon 1991, 1994) have not controlled for maternal effects arising from mother's quality or litter *per se*. Nevertheless, in general the findings seem to support the view that large size in small mammals is beneficial. For example, Mappes et al. (1995) showed that the probability of *Clethrionomys glareolus* females starting to breed during the summer of their birth increases with the body weight at weaning. Furthermore, in prairie voles *Microtus ochrogaster* higher weaning weight enhances future survival and breeding success (Solomon 1991, 1994). In the present study, the weight of offspring at weaning was not only affected by the treatment, but also by the birth weight of pups. This was true even when cross-fosterings were performed to randomize for prenatal maternal effects. Further, in a recent enclosure experiment (T. Mappes and E. Koskela unpublished data), birth weight of bank vole pups correlated positively with their probability to mature, and also with the size of their first litters. This suggests that influence of the prenatal environment on future performance of pups is important. Taken together, there are some indications of advantages of large size at birth and at weaning for future survival and reproductive success in small mammals. Clearly more data and experimental studies are needed to assess the significance of size for future performance.

Conclusions

Our experiment suggests that reproductive success of bank vole females is negatively density-dependent in terms of number of weanlings. Density-dependent factors do not seem to limit the nursing effort of females as there was no difference in the quality of offspring between the density treatments. The results support earlier findings of the existence of a trade-off between the number and quality of offspring in small mammals, and calls for more detailed investigations about the role of reproductive costs as determinants of optimal reproductive effort in small mammals.

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Table 1. Number of offspring after manipulation and at weaning in relation to manipulation groups. Run = study period, Density = high/low density, Litter = control/enlarged litter. Two-way ANOVA used.

	df	MS	F	p
After manipulation				
Run	3, 41	5.35	2.51	0.072
Density	1, 41	3.28	1.54	0.222
Litter	1, 41	33.80	15.84	<0.001
Litter * Density	1, 41	0.05	0.02	0.879
At weaning				
Run	3, 41	0.77	0.10	0.962
Density	1, 41	53.21	6.59	0.014
Litter	1, 41	0.50	0.06	0.805
Litter *Density	1, 41	1.33	0.17	0.687

Table 2. Number of successful (weaned at least one pup) and unsuccessful females in different treatments.
 C = control litters, E = enlarged litters.

Replicate	1		2		3		4									
	High	Low	High	Low	High	Low	High	Low								
Litter manipulation	C	E	C	E	C	E	C	E	C	E	C	E	C	E		
Successful females	4	3	3	2	2	0	2	2	0	2	2	2	2	1	1	1
Unsuccessful females	2	3	0	1	1	3	0	0	3	1	1	1	1	2	0	0
Total	6	6	3	3	3	3	2	2	3	3	3	3	3	3	1	1

Table 3. The logit models of weaning success of females in relation to density manipulation (Density) and litter manipulation (Litter).

Model	G	df	p
1) Density + Litter + Density * Litter	0.000	0	1.000
2) Density * Litter	0.036	1	0.849
3) Density	0.944	2	0.624
4) Litter	6.925	2	0.031
5) Constant	7.712	3	0.052

Table 4. Descriptive statistics about breeding parameters following the manipulations. Values represent mean \pm s.e. except for the first variable. For statistics see Table 5.

Density manipulation	High		Low	
	Control	Enlarged	Control	Enlarged
Litter manipulation				
Proportion of mothers producing second litters (n)	73.3 (15)	46.7 (15)	77.8 (9)	77.8 (9)
Litter size	5.7 \pm 0.8	7.4 \pm 0.2	7.4 \pm 0.8	7.4 \pm 0.3
Mean weight of pup at birth (g)	1.9 \pm 0.1	1.7 \pm 0.1	1.7 \pm 0.1	1.7 \pm 0.1
Female weight change (g)	5.9 \pm 1.3	3.9 \pm 1.2	4.9 \pm 0.6	4.7 \pm 0.9

Table 5. Three-way ANOVAs of litter size, female weight change and mean birth weight of pups from subsequent breeding. Success = weaning success (yes/no), Density = high/low density, Litter = control/enlarged litter. All other two-way and all three-way interactions were non-significant ($p > 0.05$).

Variable	Factor	df	F	p
Litter size	Success	1, 24	0.00	0.976
	Density	1, 24	1.86	0.185
	Litter	1, 24	0.49	0.492
	Density * Litter	1, 24	1.58	0.221
Female weight change	Success	1, 19	0.92	0.350
	Density	1, 19	0.04	0.842
	Litter	1, 19	1.00	0.329
	Density * Litter	1, 19	0.10	0.758
Mean birth weight	Success	1, 21	0.03	0.854
	Density	1, 21	0.38	0.544
	Litter	1, 21	1.77	0.198
	Density * Litter	1, 21	0.99	0.331

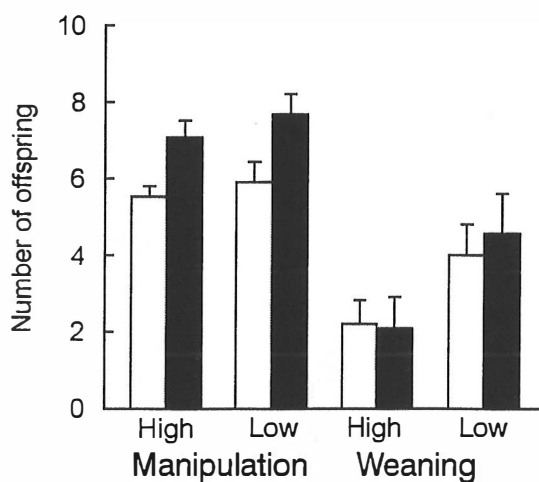


Fig. 1. Number of offspring per female after manipulation and at weaning in different treatments. High = high density treatment, Low = low density treatment, control litters = white bars, enlarged litters = black bars. Bars show the mean \pm s.e. For statistics see Table 1.

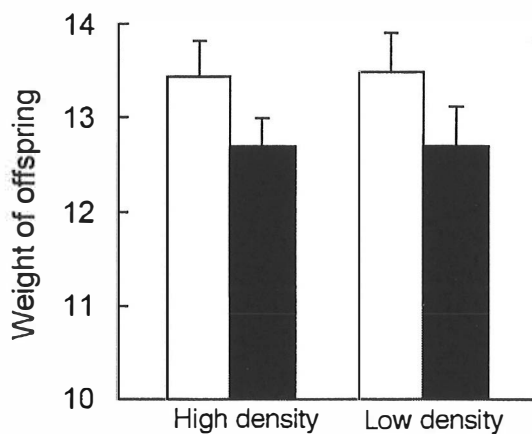


Fig. 2. Weight of 30d old offspring (in grams) in different treatments. Control litters = white bars, enlarged litters = black bars. Bars show the mean \pm s.e.

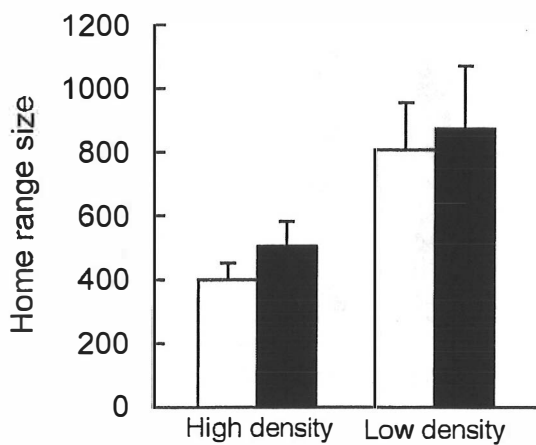


Fig. 3. Home range size of females (90% Mononuclear probability polygon, in m²) in different treatments after manipulation. Control litters = white bars, enlarged litters = black bars. Bars show the mean \pm s.e. For statistics see the text.

III

Territorial behaviour and reproductive success of bank vole *Clethrionomys glareolus* females

By

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Territorial behaviour and reproductive success of bank vole *Clethrionomys glareolus* females

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Summary

1. Although spacing behaviour in small mammals has been studied extensively, the ultimate function of territoriality in females is frequently under debate. Furthermore, almost no data exists on how territorial behaviour of females changes during reproductive cycles and how that affects their reproductive success. We studied these questions in large outdoor enclosures using the bank vole *Clethrionomys glareolus* as a study species.

2. Home range dynamics of females and behaviour of territory owners against intruder females were determined during four distinct periods of the reproductive cycle: (i) when females were non-pregnant, (ii) in early pregnancy, (iii) in late pregnancy and (iv) when females were lactating.

3. Home range size and home range overlap of females decreased from period (i) to period (iv) simultaneously when nearest neighbour distance increased. So, at the time of parturition female home range size (foraging area) was at its smallest but territory size (breeding area) at its largest.

4. Attacks of owner females increased and amicable behaviour decreased during the reproductive cycle. Attacks tended to correlate positively and amicable behaviour negatively with home range size.

5. The size of a female's home range significantly correlated with her litter size. However, the weight of females did not correlate with their home range size or litter size.

6. During lactation, nearest neighbour distance correlated negatively with the number of weanlings. Further, home range overlap did not have an effect on the number of weanlings. So, in contrast to an earlier study by Mappes et al. we did not find support for infanticide directly affecting reproductive success of females. That might be due to synchronous breeding in our study females in contrast to females in Mappes et al.

7. Our results indicate that space-resource (large home range) and thus probably food may be an important determinant of litter size in the bank voles. Aggressive defence of a territory may have a role in determining the amount of resources available for reproduction and thus the reproductive success.

8. Our study gives new evidence both for and against the two hypotheses for function of female territoriality and suggests that they are not necessarily mutually exclusive.

Key-words: home range, infanticide, reproductive cycle, space defence, spacing behaviour.

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Introduction

Territoriality of females in small mammals has been widely accepted as a means of defending a food resource (Ostfeld 1985, 1990; Ims 1987). According to this resource-defence hypothesis, food limits repro-

duction, and the distribution and type of food determines the spacing pattern of females. Thus, energy and nutrients are regarded as the most important determinants of female reproductive success, and defending a territory provides exclusive access to these resources (Ostfeld 1985). In contrast, the pup-defence

hypothesis (Wolff 1993) states that the ultimate function of territoriality is to defend a nest site aggressively and deter infanticide. Access to food may simply be a by-product of providing space exclusive from potentially infanticidal intruders.

Indirect evidence on the importance of food in spacing patterns comes both from comparative studies between populations (e.g. Andrzejewski & Mazurkiewicz 1976; Ylönen, Kojola & Viitala 1988) and field manipulations. Field studies, where access to food has been manipulated, have often shown that extra food decreases the size of the female home range and increases home range overlap between neighbouring females (e.g. Taitt & Krebs 1981; Ostfeld 1986; Ims 1987; Desy, Batzli & Liu 1990). Daily energy requirements of breeding mammals are much higher compared with non-reproductives (Gittleman & Thompson 1988). Pregnant bank voles *Clethrionomys glareolus* Schreber require an average 24% and lactating females even up to three times more energy than non-reproducing individuals (Kaczmarek 1966). So, if food is postulated as the most important resource of a territory, during a reproductive cycle (from sexual quiescence until the onset of lactation) a female should demand continually increasing food resources. That could lead to an increase in home range size and decrease in home range overlap with other individuals competing for the same resources. However, the relationship between the phase of female reproductive cycle and spacing behaviour in the field is largely unknown (Madison 1978; Gipps 1985). In fact, according to Wolff (1993) there is no evidence that female home range size during the reproductive season is based on energy demands.

Studies examining spacing behaviour of microtine females and threat of infanticide are quite rare. In our field observations and enclosure experiments (H. Ylönen, E. Koskela and T. Mappes, unpublished data) we have observed infanticide between *C. glareolus* females. As infanticide has sometimes been neglected as a phenomenon affecting spacing behaviour and reproductive success of reproducing females (Wolff 1993), it should receive more attention. Space resource can also mean (other than just food) an exclusive area to decrease the probability of intruders entering the nesting areas. Females should defend their territories most intensively when pups are present, and in that time females should have the largest amount of exclusive space from other females. So, also according to the pup-defence hypothesis, female spacing behaviour should change during the reproductive cycle.

Determinants of the reproductive success of female voles have seldom been studied. Breeding success has been estimated based on the number of pregnancies during the breeding season (e.g. Gliwicz 1990) or, more usually, by determining the number of weanlings (e.g. Mappes, Ylönen & Viitala 1995b). However, there is a shortage of data where the characteristics of

voles in a field population (e.g. age, reproductive status, density, kinship) are under control and the spacing behaviour and reproductive success of individuals can be determined.

In the *C. glareolus*, mature females have been observed to be territorial while male home ranges are larger and overlapping (Mazurkiewicz 1971; Bujalska 1973). Indeed, possession of a territory and thus of access to its space and nutrient resources is a prerequisite for breeding in *Clethrionomys* females (Kalela 1957; Viitala 1977; Saitoh 1981; Bujalska 1985; Kawata 1987). We used the bank vole as a study species to examine how female home range size, home range overlap and territorial behaviour change during the reproductive cycle, and how these factors affect the reproductive success of females. Further, we discuss our results with reference to the pup-defence hypotheses as explanations of female territoriality. In this paper we define home range as a foraging area, usually overlapping, whereas territory is part of home range and mutually exclusive apart from other females. Territory could also be considered as a breeding area. These definitions are in accordance with the general definition of female territoriality in small mammals (Kaufmann 1983) and that used in the bank vole (Bujalska 1991).

Methods

STUDY SITE AND ENCLOSURES

The study was conducted during July–August 1994 at Konnevesi, central Finland (62°37'N, 26°20'E). Vole populations were established on seven 0.25-ha enclosures in a homogeneous field abandoned from agriculture 10 years ago. The vegetation in the enclosures was typical for old fields: many grasses (e.g. *Alopecurus pratensis* L., *Phleum pratense* L., *Elymus repens* (L.) Gould, *Deschampsia* Beauv. spp., *Poa* L. spp.) and herbs (e.g. *Ranunculus acris* L., *Hypericum maculatum* Crantz, *Geum rivale* L., *Alchemilla* L. spp., *Trifolium* L. spp.) dominated the field layer. There were also some saplings of *Alnus incana* (L.) Moench and *Salix* L. spp.

The fences were constructed by embedding 1.5-m galvanized sheet metal into the ground to a depth of about 0.5 m, giving a wall height of 1 m. The fence prevented the movement of voles well, not a single vole immigrated into the enclosures from surrounding fields and forest during the study. For monitoring the voles 25 multiple-capture live traps were used in each enclosure with 10 m between the trap stations. Each trap was covered by a galvanized sheet metal chimney that reduced exposure to temperature extremes.

STUDY ANIMALS AND METHODS

Overwintered female *C. glareolus* ($n = 35$) at the same reproductive status (mature, non-pregnant but having

given birth once earlier in the summer) were used in the study. All voles for the study, caught from nearby forests in the early spring were randomly assigned to the seven enclosures taking into account that they originated from different areas to ensure that all individuals were unrelated and unfamiliar with each other. There were no differences in the weights of females in different enclosures at the beginning of the study (one-way ANOVA, $P > 0.9$). Five females (individually marked) were released simultaneously in the middle of each enclosure on day 1 of the study. This density is comparable to the highest breeding densities observed in earlier studies (Bujalska 1970; Ylönen *et al.* 1988). Three mature, randomly chosen males were introduced to each enclosure ($n = 21$) on day 11.

For monitoring the populations there were five trapping periods in relation to the reproductive state of females: (i) when non-pregnant; (ii) in early pregnancy; (iii) in late pregnancy; (iv) when lactating; (v) when weanlings were recruited to the population (Table 1). Each trapping period consisted of 10 trap checkings, twice a day (morning and evening) for 5 days except for the two last periods, when trap checkings were done three times a day. In this way we tried to minimize the possible harmful effects of longer trap-checking intervals for pups and/or lactating females. At each capture the following variables were recorded for an individual vole: identity, sex, trap location, weight and reproductive status. All voles were released at the point of capture. During the study some females disappeared from enclosures; they had most probably died. By immediately introducing new females in the same reproductive condition from laboratory stock (consisting of animals trapped from the same area as all the other study animals), it was ensured that there was always a constant density of females in each enclosure. However, only females who were present throughout the study and successfully gave birth ($n = 25$) were used in the analyses. These females were distributed in the seven enclosures as follows: 2, 4, 3, 3, 3, 5, 5 females in enclosures 1–7, respectively.

After the third trapping period (late pregnancy) all females ($n = 35$) were removed from enclosures to standard breeding cages in the laboratory, where they gave birth. By inspecting females twice a day the actual litter size at parturition was determined. The breeding of females within each enclosure was in close synchrony: all gave birth within 2–3 days. Females and their litters (pups marked) were returned (still in breeding cages) to enclosures and placed in an activity centre, which should lie very near to their nests (Mironov 1990). Cages were left open so that the mothers could carry the pups back to the nests themselves. The advantages of this method are that actual time of parturition is known and the number of pups born can be compared with the number of weanlings.

Home range size, nearest neighbour distance and activity centre were estimated for individual females separately for each trapping period. Home ranges were calculated using two different methods: minimum convex polygon (MCP) and 80% mononuclear probability polygon centred on arithmetic mean (80% MPP) (Kenward 1987). This was to make comparisons of space use easier between different studies, as suggested for example in Andreassen *et al.* (1993). Both home range estimates are presented but only MCP is used in all analyses (except in Fig. 1). This was because we wanted to include all the possible areas in our home range estimates that females may have used for foraging. The number of captures per 10-trap checkings (trappability) differed between trapping periods (mean and variance for trapping periods 1–4: 7.8 (0.3), 8.6 (0.2), 8.1 (0.2) and 6.9 (0.2), repeated measures MANOVA; enclosure: $F = 0.6$, $P = 0.724$; female state: $F = 9.03$, $P = 0.001$). This was due to low trappability in daytime during the fourth trapping period (when traps were checked three times a day); trappability did not differ between first three trapping periods ($P = \text{NS}$). Home range size (MCP) was not correlated with the number of captures ($r_s = 0.09$, $P > 0.7$, $r_s = 0.32$, $P > 0.1$, $r_s = 0.07$, $P > 0.07$, $r_s = 0.20$, $P > 0.3$, trapping periods 1–4, respectively).

Table 1. Study design and reproductive cycle of females

Day	Study design	Reproductive cycle
1	Introduction of females	Habituation and occupation of territories
6–10	First trapping period First behavioural trials	Females not breeding
11	Introduction of males	Mating
15–19	Second trapping Second behavioural trials	Females in early pregnancy
24–28	Third trapping period Third behavioural trials	Females in late pregnancy
28–32	Determination of litter size	Parturition
42–45	Fourth trapping period Fourth behavioural trials	Females with litters, lactating
60–62	Fifth trapping period Determination of number of weanlings	Juveniles at the age of one month

The activity centre was calculated as the arithmetic mean point of each individual's capture coordinates (Hayne 1949). Nearest neighbour distance was calculated from distances between the activity centres of neighbouring females. Percentage home range overlap was measured as the proportion of total trap sites in the home range of each female also visited by other females (Ims 1987; Mappes *et al.* 1995b). This estimate does not take into account the frequency of use of space in the area of overlap, as does, for example Smith & Dobson's (1994) weighted overlap value. However, we suggest that the value we use is more appropriate for the purposes of the present study because it gives the accurate proportion of exclusiveness of a home range.

DETERMINATION OF TERRITORIAL BEHAVIOUR

The behaviour of owner females against intruder females was determined four times, once for each trapping period during the last 2 days of trapping (Table 1). Each owner was tested only once against an intruder during each reproductive phase. We used a total of 58 mature non-pregnant intruder females originating from field or laboratory stock. Every intruder was used only once in a trial with each owner and the same intruder was used only in one experiment per day. The weights of intruder females did not differ between trapping periods ($P > 0.10$). Behavioural trials lasted 10 min and were carried out in small arenas (50 × 60 cm) with wiremesh floors and Plexiglas front walls. These arenas were placed directly on the ground at the trap station nearest to the activity centre of the female, with the prerequisite that the owner female had visited that particular site during the trapping period. Both owner female and intruder were kept in separate tubes on opposite sides of the arena for 1–2 min before the start of the trial. We used the description of female behaviour following Ims (1987), where recorded variables were: amicable behaviour, approaching, freezing, avoidance, fleeing, threat and attacks. The frequencies of all mutually exclusive variables were counted continuously throughout the trial. Immediately after each trial the owner female was released at the place of capture and the intruder was returned to laboratory. These kinds of behavioural observations, which are based on the assumption that the owner female senses her situation in the territory by the means of olfactory cues from the surrounding environment, have been verified to operate well in previous studies (e.g. Wolff, Freeberg & Dueser 1983; Ims 1987).

DATA ANALYSIS

Because it could be expected that the change of variables (home range size, overlap, nearest neighbour distance, behaviour) is a function of female state, repeated measures MANOVA was used for making com-

parisons between different trapping periods. Enclosure was used as a separate factor in these analyses. Trappability was used as a varying covariate in repeated measures analyses of space use. Linear term of polynomial contrast was significant for all significant changes in behaviours (except for avoidance) and is reported. When analysing female behaviour, we used the relative proportion of different behavioural categories from the total behaviour of each female during that particular trial. These behavioural variables and overlap variable were arcsine square root transformed before analysis.

After females had given birth in the laboratory, their litter size was manipulated as a subject for a separate study (Mappes, Koskela & Ylönen 1995a). The only dependent variable used in the present study affected by the manipulation was the number of weanlings (when using $P = 0.10$ as a significance level). In correlation analyses concerning the number of weanlings, Kendall's partial correlations were used with manipulation as a controlling variable. For the other correlation analyses, Spearman rank correlations were used. The sample size was 25 in all analyses except when otherwise mentioned. All the tests are two-tailed. The statistical analyses were performed by using SPSS for Windows (SPSS Inc.; Norusis 1992).

Results

SPACE USE AND REPRODUCTIVE SUCCESS

The home range size of females decreased significantly during the reproductive cycle according to the MCP estimate, and tended to decrease according to the 80% MPP estimate (MCP: enclosure: $F = 0.76$, $P = 0.608$; covariate: $t = 0.582$, $P = 0.568$; female state: $F = 3.12$, $P = 0.034$; 80% MPP: enclosure: $F = 1.34$, $P = 0.293$; covariate: $t = 0.893$, $P = 0.385$; female state: $F = 2.41$, $P = 0.077$; Fig. 1). The home range overlap also decreased during the study (enclosure:

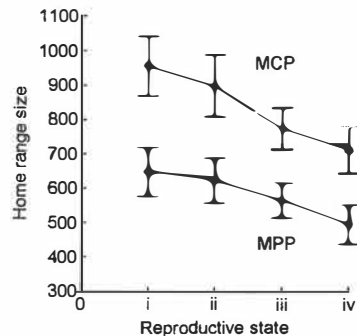


Fig. 1. Home range size (in m^2) of females calculated as minimum convex polygons (MCP) and 80% mononuclear probability polygons (MPP) in different reproductive states. (i) = non-pregnant; (ii) = early pregnancy; (iii) = late pregnancy; (iv) = lactating. Bars show the mean \pm standard error. For statistics see the Results.

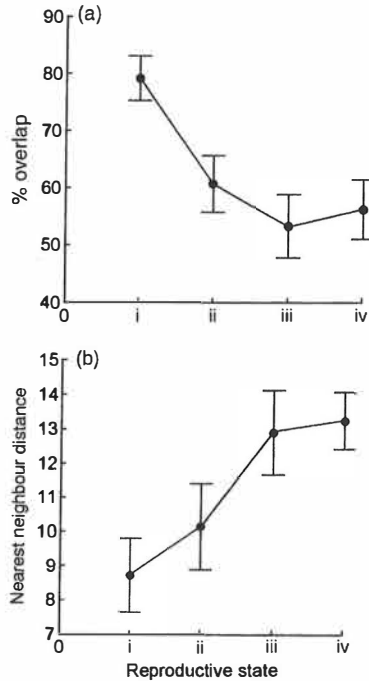


Fig. 2. Proportional home range overlap (a) and nearest neighbour distance (in m) (b) of females during reproductive cycle. See legend of Fig. 1 for details.

$F = 0.76$, $P = 0.608$; covariate: $t = -0.873$, $P = 0.395$; female state: $F = 7.58$, $P = 0.000$; Fig. 2a). Nearest neighbour distance increased as the study proceeded (enclosure: $F = 6.83$, $P = 0.001$; covariate: -1.236 , $P = 0.233$; female state: $F_{(3,16)} = 3.29$, $P = 0.048$; Fig. 2b).

Female weight at the beginning of the study or post-partum weight did not correlate significantly with home range size during any of the trapping periods (Fig. 3 for late pregnancy). The correlation coefficients between initial weight and post-partum weight of females with litter size were also non-significant (initial: $r_s = 0.269$, $P = 0.194$; post-partum:

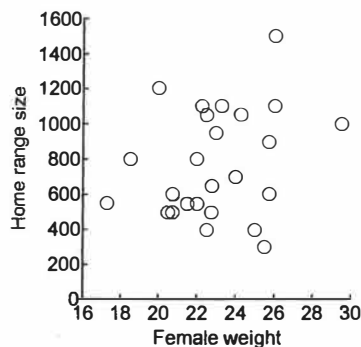


Fig. 3. The relationship between initial weight of female (in g) and home range size (in m^2) at late pregnancy ($r_s = 0.239$, $P = 0.249$).

$r_s = 0.194$, $P = 0.352$). The size of female home ranges at late pregnancy significantly explained the variation in litter size (Fig. 4). Litter size at birth correlated significantly with the number of weanlings ($r_k = 0.544$, $n = 24$, $P = 0.000$). Home range sizes at late pregnancy and at lactation also correlated significantly ($r_s = 0.699$, $P = 0.000$). This most probably leads to the result that when females were nursing their young, their home range size correlated positively with the number of weanlings ($r_k = 0.399$, $n = 24$, $P = 0.006$). However, the number of weanlings correlated negatively with nearest neighbour distance ($r_k = -0.343$, $n = 24$, $P = 0.019$). The home range overlap did not have an effect on the number of weanlings ($r_k = -0.079$, $n = 24$, $P = 0.588$).

BEHAVIOUR

When the time for parturition approached, females increased their attacks towards intruders significantly (Table 2). Simultaneously, the proportion of amicable behaviour decreased (Table 2). The linear term of the polynomial contrast was highly significant for both variables (attacks: $F_{(1,18)} = 14.01$, $P = 0.001$; amicable behaviour: $F_{(1,18)} = 49.54$, $P = 0.000$). Avoidance by owners also varied during the study (Table 2). Intruder behaviour did not change (in all cases $P > 0.05$) except for fleeing, which increased as the study proceeded (enclosure: $F_{(6,18)} = 2.23$, $P = 0.088$; female state: $F_{(3,16)} = 12.11$, $P = 0.000$; linear term: $F_{(1,18)} = 6.04$, $P = 0.024$). This change was most probably due to increased attacks of owners towards intruders.

Home range size at late pregnancy tended to correlate positively with attacks ($r_s = 0.374$, $P = 0.065$) and negatively with amicable behaviour ($r_s = -0.350$, $P = 0.087$). Threats had a negative correlation with home range size ($r_s = -0.466$, $P = 0.019$) when females were nursing their young. In the behavioural trials the threatening female was usually in the corner of the arena defending herself as another female tried

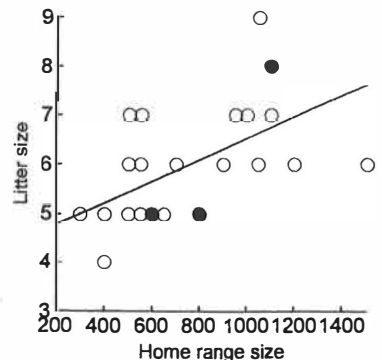


Fig. 4. Linear regression between female home range size (in m^2) at late pregnancy and litter size. Filled dots indicate two cases. $F = 10.02$, $n = 25$, $P = 0.004$; equation: litter size = $4.356 + 0.002 \times$ home range size.

Table 2. Observed proportion of different behaviours of owner females against intruder females in relation to reproductive cycle. First line of each variable gives statistics for female state, second for enclosure. Sample size is 25 for all behaviours. Values represent mean \pm standard error

	Non-pregnant	Early pregnancy	Late pregnancy	Lactation	<i>F</i> *	d.f.	<i>P</i>
Amicable behaviour	9.8 \pm 2.5	8.9 \pm 2.2	1.3 \pm 0.8	0.7 \pm 0.4	25.91	3,16	0.000
					2.50	6,18	0.062
Approach	42.9 \pm 4.6	53.3 \pm 5.1	62.1 \pm 7.0	57.1 \pm 5.4	1.71	3,16	0.206
					1.05	6,18	0.430
Freeze	1.1 \pm 0.8	1.9 \pm 1.4	0†	0†	0.00	1,18	0.983
					1.15	6,18	0.376
Avoid	22.3 \pm 4.9	16.7 \pm 4.4	7.4 \pm 2.3	14.5 \pm 4.6	3.74	3,16	0.033
					0.64	6,18	0.700
Flee	0.6 \pm 0.6	0.3 \pm 0.3	0†	0.0 \pm 0.0‡	0.04	2,17	0.959
					1.26	6,18	0.325
Threat	21.1 \pm 4.0	15.4 \pm 4.7	21.0 \pm 5.7	10.5 \pm 4.2	0.70	3,16	0.565
					0.63	6,18	0.708
Attack	2.2 \pm 1.0	3.6 \pm 1.9	8.3 \pm 4.4	16.9 \pm 4.9	5.03	3,16	0.012
					0.28	6,18	0.940

* Repeated measures MANOVA used.

† No cases for this variable.

‡ Proportion less than 0.0%.

to chase her away. That also indicates the result that threats correlated negatively with approaching ($r_s = -0.639$, $P = 0.001$). Female amicable behaviour at late pregnancy correlated negatively with their litter size ($r_s = -0.461$, $P = 0.021$).

Discussion

In comparative field studies of female territoriality there are usually many factors that make it difficult to interpret the results, e.g. density-dependent plasticity in spacing patterns and kin group effect (Wolff 1993). The former is caused by environmental conditions, mainly by variability in the food resource and in number and sexual status of individuals in a population. The effects of these factors on spacing systems are reported in several studies (reviewed in Bondrup-Nielsen & Karlsson 1985; Ylönen 1990). The latter, the kin group effect, is caused by greater overlapping of home ranges in related females compared with non-kin (Ims 1989; Lambin & Krebs 1993; Mappes *et al.* 1995b). Because the voles used in the present study were non-kin and unfamiliar to each other and the density in enclosures was the same during the study, these factors did not affect this study. Changing environment (seasonality) is one possible factor affecting our results. However, because changes in the spacing behaviour of females were so remarkable and were observed in midsummer during a rather short period (less than 40 days), we suggest seasonality as an unlikely cause of the observed changes in spacing behaviour of females. We cannot rule out the possible effect of changing food resources on our results. However, bank voles seem to have a rather continuous and stable availability of food. They use a varied range of food items from leaves, stems and seeds of plants to fungi, berries and invertebrates (e.g. Hansson 1971).

Because most of these are also highly renewable during the breeding season, we assume that food resources remained relatively constant during the study and that any possible changes were spatially quite similar.

SPACING BEHAVIOUR IN RELATION TO REPRODUCTIVE STATE

According to our study, female aggression increases significantly and amicable behaviour decreases as the time for parturition gets closer. These results agree with an earlier laboratory experiment with the bank voles (Rozenfeld & Denoël 1994) and the finding that aggressiveness increases at the onset of lactation in microtines (e.g. Ayer & Whitsett 1980; Mallory & Brooks 1980). At the same time, the size of home ranges gets smaller, the overlap between home ranges decreases and home range centres (activity centres) move further from each other. Clearly, the spacing pattern of females develops towards strict breeding territoriality. When studying home range oscillations in relation to female density in *Microtus pennsylvanicus*, McShea (1989) suggested that changes in home range size of females after parturition are more dependent on interactions with other individuals in the population than solely caused by mother-offspring interactions. Also, Korn (1982) failed to find a relationship between energetic requirements of bank vole females and the size of their home range and concluded that behavioural aspects seem to be a more important determinant of home range size. Our study, where individual females were followed throughout their reproductive cycle, gives support for these suggestions.

FEMALE BEHAVIOUR AND REPRODUCTIVE
SUCCESS

In our study female home range size at late pregnancy significantly correlated with her litter size. This result agrees with a recent experiment (E. Koskela, T. Mappes and H. Ylönen, unpublished data). One must remember that infanticide could not affect litter sizes because they were determined in the laboratory. Because the weights of females did not correlate with litter or home range size (even though considerable variation occurred in weights; Fig. 3), it seems that purely physiological properties (e.g. dominance status via large size) are not sufficient to explain our result. Furthermore, all the females were of the same age and sexual status, so the sizes of home ranges and litters seem to depend on other characteristics of females, behaviour being particularly important. According to the laboratory study of Rozenfeld & Denoël (1994), aggressiveness is a crucial part of the spacing behaviour of breeding females. It has been suggested that in the field scent marking by territory owners functions as a more usual form of territorial defence than aggression (Viitala & Hoffmeyer 1985). However, our behavioural trials revealed a positive correlation between aggressive behaviour (attacks) and home range size. Also, both threats (submissive act) and home range size, as well as amicable behaviour and litter size, correlated negatively. According to our results females who were aggressive towards intruders had larger home ranges. The larger home range may provide more food, which has been shown to affect litter sizes in many microtines (Hoffmann 1958; Batzli & Pitelka 1971; Cole & Batzli 1978). However, experiments are needed to certify the possible link between female behaviour, home range size and reproductive success.

INFANTICIDE AND REPRODUCTIVE SUCCESS

Infanticide is already known to occur in some microtines, and recent experimental studies (e.g. Cicirello & Wolff 1990; Wolff & Cicirello 1991; Mappes *et al.* 1995b) and theoretical papers (Wolff 1993) indicate that this phenomenon might also exist in the bank vole. In fact, in recent enclosure experiments (H. Ylönen, E. Koskela and T. Mappes, unpublished data) we have observed infanticide between female bank voles. So, as we now suggest that infantile behaviour exists in the bank vole, the remaining questions are (i) 'Does infanticide directly affect the reproductive success of females?' and (ii) 'Does threat of infanticide affect the spacing behaviour of females?' In the present study, we observed infanticide only indirectly, comparing the number of weanlings with the nearest neighbour distance and home range overlap among mothers. In the study by Mappes *et al.* (1995b) they found a significant positive correlation between nearest neighbour distance and number of weanlings. They

suggest that this might be due to neighbours killing each others' juveniles. However, in the present study, this correlation was negative, not supporting earlier results. Also, home range overlap did not affect the number of weanlings. Our findings suggest that, in the present study, reproductive success of females was not affected directly by infanticide. This difference between our results and those by Mappes *et al.* (1995b) may be due to the different timing of breeding in females. In the present study all females were breeding in synchrony and it might be possible that they had limited opportunity or potential for infanticide, as suggested in Lambin (1993). If, however, breeding is asynchronous (as in Mappes *et al.* 1995b), there are continuously females in different reproductive states and vulnerable pups in the population that may promote occurrence of infanticide.

Conclusions

Our study provides new data showing that the spacing behaviour of bank vole females is specific to the different phases of the reproductive cycle: at the same time as aggressive behaviour increases and amicable behaviour decreases, the spacing pattern of females develops towards strict breeding territoriality. At the time of parturition female home range size (foraging area) is at its smallest but territory size (breeding area) is at its largest. The ability to defend the territory aggressively may have an important role in determining the amount of resources available for reproduction (size of a home range) and is further reflected in the reproductive success. Infanticide did not seem to affect breeding success of females when they were breeding in synchrony. All these findings may also correspond to other small mammals, where females are territorial when breeding.

Our study provides new evidence both for and against the hypotheses of territoriality in female small mammals. On the one hand, that the size of home range, behavioural characteristics of females and their litter size seem to be linked emphasizes the importance of food as the cause of territoriality. However, the size of home range decreased during the reproductive cycle, in contradiction to the increased food demands of females. On the other hand, the finding that in territorially breeding voles like *Clethrionomys glareolus* the home range becomes more exclusive from other females when pups are present supports the pup-defence hypothesis (Bujalska 1991; Wolff 1993). Further support comes from the fact that females defend their territories most intensively when pups are present. However, that is also the time when food demands are greatest for mothers. We did not find evidence that infanticide affected the reproductive success of females in the present study. Our study suggests that food-defence and pup-defence hypotheses for female territoriality are not necessarily mutually

exclusive and demonstrates the need for experimental studies.

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IV

Offspring growth, survival and reproductive success in the bank vole: a litter size manipulation experiment

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OFFSPRING GROWTH, SURVIVAL AND REPRODUCTIVE SUCCESS IN THE BANK VOLE: A LITTER SIZE MANIPULATION EXPERIMENT

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ABSTRACT

To estimate the optimality of brood size, it is essential to study the effects of brood size manipulation on offspring survival and reproductive success. Moreover, testing the generality of the hypothesis of reproductive costs requires experimental data from diversity of organisms. Here I present data on growth, survival and reproductive success of bank vole *Clethrionomys glareolus* individuals from manipulated litters. Furthermore, the survival of mothers whose litter size was manipulated was studied. At weaning, the mean weight of pups from enlarged litters was lower and from reduced litters higher compared to control litters. After winter, at the start of breeding season individuals from enlarged litters, especially males, were still lighter than individuals from the other two treatments. Litter enlargements did not increase the number of reproducing female offspring per mother, neither the litter sizes of female offspring differed between treatments. There were no differences in winter survival of offspring between treatments after weaning, but among female offspring weaning weight explained the survival probabilities over winter. Higher weight of females at winter determined the probability to start reproducing at spring. The survival of mothers did not seem to differ in relation to litter manipulation performed previous year. According to the results mothers nursing enlarged or reduced litters do not gain any fitness benefits in terms of number of surviving offspring until breeding. The results are consistent with the majority of experiments conducted in birds, that have found costs of enlarged brood appearing as offspring trade-offs rather than as parent trade-offs.

INTRODUCTION

Trade-offs between life history traits as determinants of reproductive effort have been traditionally studied using brood size manipulations. In these experiments costs of reproduction have most often been found to arise as offspring trade-offs than parent trade-offs (reviewed in Lindén & Møller 1989, Roff 1992). This seems reasonable because lifetime reproductive success of females depends more on long-term survival than fecundity during current breeding attempt in most species. However, the importance of offspring trade-offs is surprisingly poorly investigated (Roff 1992). To be able to estimate the selective advantage or disadvantage of enlarged brood size the subsequent

survival and reproductive success of offspring should be studied. These kind of studies are often difficult to proceed because offspring may settle to breed very far from their natal site (but see e.g. Gustafsson & Sutherland 1988).

Previously the hypothesis of reproductive costs (Williams 1966) has been experimentally studied almost exclusively using birds (for reviews see Lindén & Møller 1989, Dijkstra et al. 1990, Roff 1992, Stearns 1992). However, testing the generality of the hypothesis would require experimental data on a diversity of organisms with e.g. different forms of parental care. Until now, there are only two studies in mammals where costs of reproduction and optimal litter size have been experimentally studied in the field (Hare & Murie 1992, Mappes et al. 1995). Further, only in the experiment by Mappes et al. (1995) litter size manipulations were proceeded taking into account the original litter size of mothers. This is essential to be able to control for differences in individual circumstances which may affect the results (e.g. individual optimization hypothesis, Perrins & Moss 1975, Morris 1985). In general, intergenerational trade-offs between life-history traits (e.g. between reproductive effort of a female and the future survival of her offspring) have received much less attention than intragenerational trade-offs (e.g. between reproductive effort of female and her future survival) (Stearns 1992). This is especially true in mammals; e.g. the survival of manipulated offspring until their first breeding opportunity or subsequent reproductive success have not been studied earlier. Thus, the evidence evaluating the hypothesis of reproductive costs, particularly on subsequent performance of manipulated offspring, is only possible to generalise to avian populations.

Here I report the results of a litter manipulation experiment where both intra- and intergenerational trade-offs were studied using the bank vole. The study extends the litter manipulation experiment by Mappes et al. (1995). In this paper I report 1) the long-term growth and survival of manipulated offspring to their first breeding season and factors affecting subsequent breeding success. Furthermore, 2) the possible survival costs of litter size manipulation for mothers were studied.

MATERIAL AND METHODS

Study site and animals

The study was conducted in 1994-1995 at Konnevesi, central Finland (62°37'N, 26°20'E) in eight 0.25 ha outdoor enclosures. For monitoring the populations there were 25 multiple capture live traps in a 5 x 5 array in each enclosure with 10 meters between the trap stations. For a description of enclosures and the habitat see Koskela et al. (1997). The animals for the study originated from an litter manipulation experiment (see Mappes et al. 1995). Each enclosure population consisted of individuals with two different origin: 1) Mothers, whose litter sizes were manipulated during summer 1994 (see later in text) and 2) non-reproducing offspring (both sexes) from manipulated litters, born at late summer 1994.

Methods

Mothers ($n = 40$, five per enclosure) were released in enclosures at the beginning of the litter manipulation study in July 1994 (Mappes et al. 1995). Before parturitions all the females were captured and transferred to the laboratory where litter manipulations took place when pups were 1-2 days old. There were three litter manipulation groups: reduced litters, 'R', with two pups removed; enlarged litters, 'E', with two pups added; and control litters, 'C', where original litter size was not changed but two pups were exchanged with another litter. Litter size manipulation groups were randomly assigned according to enclosures and original litter size categories of females (Mappes et al. 1995). Furthermore, the weight or other conditions of mothers did not differ between the treatments (Mappes et al. 1995). To be able to relate the offspring to their mothers, the pups within the litter were marked with the same code right after birth (one toe clipped off). At first capture in the enclosures, pups were given individual marks.

After the manipulation, females with their pups were released back to their territories. The sex of pups was determined at weaning and the sex ratio did not differ between treatments (percentage males:females 56:44, 54:46, 53:47 in R, C and E litters, respectively, Pearson's $\chi^2 = 0.15$, $df = 2$, $p = 0.927$). The number of offspring in each litter manipulation group did not differ between enclosures after manipulation (Pearson's $\chi^2 = 16.58$, $df = 14$, $p = 0.280$). The mean density of offspring in enclosures after manipulation was 27.8 (SE = 1.6) but already at weaning the density has decreased to in average 19.3 (SE = 2.3) offspring per enclosure.

Individuals were monitored six times during the study using live trapping. There were three trapping sessions before winter in 1994: 1) after weaning, offspring about one month old (23-25 August); 2) at the end of the breeding season (14-16 September) and 3) just before the winter, offspring about three months old (12-14 October). Another three trappings were performed from winter until spring: 4) In early winter (5-8 December), 5) at winter (7-9 February) and 6) at the beginning of breeding season (starting 8 May). During the three first trapping periods traps were checked ten times at 6-9 h intervals. At winter (trapping periods 4 & 5) only eight trap checks were made. During the last period trapping was continued until all individuals were captured from enclosures. Individuals were transferred to standard breeding cages in the laboratory where they were weighed and possible breeding of female offspring was observed. Possession of a territory is a prerequisite for reproduction in bank vole females (Bujalska 1985). Thus, the females that were pregnant at capture had been successful in competition for space with the other females in the enclosure population.

During the fourth trapping period (December) two unmarked bank voles and several field voles *Microtus agrestis* were captured from one enclosure indicating that the surrounding fence has broken. Therefore, all the data after December from that particular enclosure is omitted from analyses. The fences not only enclosed the experimental populations, they also prevented the access

of main predators (small mustelids least weasel *Mustela nivalis* and stoat *Mustela erminea*) to the enclosures.

Data analysis

In each enclosure mothers (and their offspring) from every treatment were represented. That was to control for possible environment effects, related to differences e.g. in microhabitat or snow cover between enclosures. Thus, the study design allows to simplify the analyses and the enclosure factor was omitted. The survival of offspring was studied in two ways: 1) the change in litter size of mothers during the study with repeated measures analyses and 2) the factors affecting survival of independent offspring after weaning with logistic regression. All tests are two-tailed.

RESULTS

Growth and size of offspring

At weaning, the mean weight of offspring was significantly lower in the E (enlarged) litters and higher in the R (reduced) litters compared to the control group (Fig. 1, Mappes et al. 1995). From weaning to late autumn (October) individuals in all treatments gained weight but the differences between manipulation groups remained (Repeated measures MANOVA; Time: $F_{(2,24)} = 115.41$, $p < 0.001$, Treatment: $F_{(2,25)} = 10.22$, $p = 0.001$, Interaction: $F_{(4,50)} = 0.92$, $p = 0.458$). In pair-wise comparisons both treatment groups differed significantly from the control group (R vs. C: $t = -2.23$, $p = 0.035$, E vs. C: $t = 2.23$, $p = 0.035$). Individuals lost weight until winter but already in February they started to gain weight again (Fig. 1). At the start of the breeding season the weight of offspring differed significantly between treatment groups (One-way ANOVA, $F_{(2,58)} = 6.17$, $p = 0.004$) so that individuals from E litters were significantly smaller than individuals from C and R litters (Student-Newman-Keuls, both $p < 0.05$). This was true especially in males (mean \pm SE, R litters: $25.1 \pm 0.8g$, C litters: $24.4 \pm 0.4g$, E litters: $22.8 \pm 0.7g$, One-way ANOVA: $F_{(2,29)} = 3.67$, $p = 0.038$, Duncan test: R litters significantly different from C and E litters), in females the differences in weights between treatments were only significant on $\alpha = 0.1$ level (R litters: $24.9 \pm 0.9g$, C litters: $24.9 \pm 0.6g$, E litters: $22.1 \pm 1.3g$, One-way ANOVA: $F_{(2,26)} = 2.82$, $p = 0.078$).

Survival of offspring

Litter enlargement did not increase the number of surviving offspring per mother to weaning. The number of offspring at weaning did not differ significantly between the E and C litters but was significantly lower for R litters compared to E and C litters (Fig. 2, Mappes et al. 1995). Before winter, from weaning to late autumn (August to October) the mean litter size of mothers decreased significantly in all treatments (Repeated measures MANOVA; Time: $F_{(2, 34)} = 12.19$, $p < 0.001$, Treatment: $F_{(2, 35)} = 4.20$, $p = 0.023$, Interaction: $F_{(4,70)} =$

0.37, $p = 0.830$, Fig. 2). In pair-wise comparisons between litter manipulation groups the R litters differed significantly from C and E litters (R vs. C: $t = 2.24$, $p = 0.032$, R vs. E: $t = 2.63$, $p = 0.013$, C vs. E: $t = -0.31$, $p = 0.760$). From early winter to beginning of breeding season (December to May) the mean number of offspring alive continued to decrease significantly in all treatments (Repeated measures MANOVA; Time: $F_{(2,29)} = 10.73$, $p < 0.001$, Treatment: $F_{(2,30)} = 3.46$, $p = 0.044$, Interaction: $F_{(4,60)} = 0.89$, $p = 0.474$). During this time reduced litters differed significantly only from control group (R vs. C: $t = 2.43$, $p = 0.021$, E vs. C: $t = 0.56$, $p = 0.583$, R vs. E: $t = 1.90$, $p = 0.068$). So, in May the mean number of offspring alive tended to be highest in the control group (One-way ANOVA, $F_{(2,30)} = 3.09$, $p = 0.060$, Fig. 2). This difference was significant in males (Males: mean \pm SE, R mothers: 0.5 ± 0.2 , C mothers: 1.7 ± 0.5 , E mothers: 1.4 ± 0.4 , One-way ANOVA, $F_{(2,30)} = 3.32$, $p = 0.050$, Duncan test: R litters significantly different from C litters, Females: R: 0.7 ± 0.3 , C: 1.3 ± 0.6 , E: 1.0 ± 0.4 , One-way ANOVA, $F_{(2,30)} = 0.62$, $p = 0.543$).

There was no difference in the survival of offspring from weaning to the beginning of the next breeding season between manipulation groups ($\chi^2 = 1.43$, $df = 2$, $p = 0.489$) or sexes ($\chi^2 = 0.90$, $df = 1$, $p = 0.341$) (Table 1). Males were heavier than females at weaning (mean \pm SE, Males: 12.5 ± 0.3 , Females: 11.5 ± 0.2 , Two-sample t-test $t = -3.01$, $df = 143.4$, $p = 0.003$). Survival of offspring (from weaning to breeding season) was studied further with a logistic regression model (stepwise backward procedure, SPSS Inc. 1992) with factors weaning weight, manipulation and sex. The resulting model consisted of factors sex, weaning weight and their interaction term demonstrating that weaning weight affected the survival of females and males differently. Table 1 gives the significances of logistic regression models with weight and weight by manipulation interaction separately for both sexes. In females the model with the best fit on the data consisted only of the term weight, indicating that larger female offspring were more likely to survive independently of manipulation group (Fig. 3a). In males none of the models fit the data significantly, indicating that their survival could not be explained with weaning weight or manipulation group (Fig. 3b).

Number of dead offspring did not correlate significantly with the original litter sizes of mothers until weaning (Mappes et al. 1995) or from weaning to next spring (R litters: $r_s = 0.23$, $n = 14$, $p = 0.419$, C litters: $r_s = -0.20$, $n = 9$, $p = 0.604$, E litters: $r_s = 0.07$, $n = 10$, $p = 0.839$). Neither the mother weight (postpartum) was correlated with the proportion of surviving offspring from weaning to spring (R litters: $r_s = 0.13$, $n = 10$, $p = 0.718$, C litters: $r_s = 0.20$, $n = 8$, $p = 0.639$, E litters: $r_s = 0.35$, $n = 9$, $p = 0.353$).

Reproductive success of offspring after winter

The number of reproducing female offspring (recruits) per mother did not differ between the treatments (mean \pm SE, R mothers: 0.5 ± 0.2 , C mothers: 0.7 ± 0.4 , E mothers: 0.7 ± 0.4 , One-way ANOVA, $F_{(2,30)} = 0.132$, $p = 0.877$). Neither there was difference in the proportion of breeding female offspring in May in

relation to litter size manipulation group (R litters: 70 %, $n = 10$, C litters: 50 %, $n = 12$, E litters: 70 %, $n = 10$, $\chi^2 = 1.28$, $df = 2$, $p = 0.527$). The first litter sizes of females did not differ between treatment groups (mean \pm SE, R: 5.4 ± 0.3 , C: 5.7 ± 0.7 , E: 5.7 ± 0.4 , One-way ANOVA: $F_{(2,16)} = 0.08$, $p = 0.921$). The reproductive success of male offspring was not estimated.

The weight of female offspring at weaning did not explain significantly the probability to start breeding in May (mean \pm SE, Non-reproductive: 11.0 ± 0.3 g, Reproductive: 11.8 ± 0.4 g, Logistic regression $\chi^2 = 2.35$, $df = 1$, $p = 0.125$). However, the weight of females in February, when individuals started to gain weight again (Fig. 1) explained the probability to start breeding (mean \pm SE, Non-reproducing: 15.1 ± 0.4 g, Reproducing: 16.1 ± 0.3 g, Logistic regression $\chi^2 = 5.02$, $df = 1$, $p = 0.025$).

Reproductive costs of mothers

There was no significant effect of litter size manipulation on the survival of mothers from the manipulation to the next breeding season (R mothers: 14 % survival, $n = 14$, C mothers: 22 % survival, $n = 9$, E mothers: 30% survival, $n = 10$, $\chi^2 = 0.87$, $df = 2$, $p = 0.647$). Because of low sample size this result should be treated with caution. However, the trend in survival is the opposite than what would be expected if survival costs were present. The prewinter weight of females did not differ between the treatments (Mappes et al. 1995). Female weight did not explain the probability to survive over winter (mean \pm SE, Non-surviving: 26.4 ± 1.9 g, Surviving: 27.6 ± 3.0 g, Logistic regression $\chi^2 = 1.40$, $df = 1$, $p = 0.238$). For a more detailed analysis of possible reproductive costs (e.g. fecundity costs for mothers in relation to the manipulation performed previous year, survival in relation to number of reproductive events and litter manipulation) the sample sizes are not adequate.

DISCUSSION

According to the results, nursing of a larger or smaller litter than the original litter size did not seem to increase the fitness of individual bank vole females. At the beginning of the breeding season, there was no difference in the number of reproducing female offspring or their litter size between mothers from different manipulation groups. Neither enlarging nor reducing litter size did increase the number of male offspring entering the breeding population in spring. Further, the male offspring from enlarged litters were lighter, possibly resulting in lower reproductive success in male-male competition for females (e.g. Sheridan & Tamarin 1988). No indications of survival costs were found in mothers. The results give support to individual optimization hypothesis (Morris 1985, Pettifor et al. 1988), stating that the original brood size of parents maximizes the recruitment rate of offspring.

In mammals, reproductive costs and optimal litter size have been earlier studied with unmanipulated phenotypic correlations (e.g. Millar 1973, Clutton-

Brock et al. 1982, Myers & Master 1983, Morris 1986, Murie & Dobson 1987, Boutin et al. 1988, Michener & Locklear 1990, Sikes 1995). Further, on a few litter size manipulations conducted (in laboratory: Machin & Page 1973, Fleming & Rauscher 1978, Kaufman & Kaufman 1987, in the field: Hare & Murie 1992) manipulations were carried out without taking into account the original litter size of mothers. However, the experimental approach has been suggested more reliable for detecting the costs than correlative data (Gustafsson & Sutherland 1988, Lessels 1991, Roff 1992). To my knowledge, the effect of offspring size on long-term survival and reproductive success has not been experimentally studied in mammals before. In the present study I was able to follow the long-term growth and survival of manipulated offspring to their first breeding season, and their subsequent breeding success in their natural environment. In this respect the bank vole as a study species, and small mammals in general, give some advantage over birds which often disperse soon after leaving their nest.

In birds some earlier studies have shown negative effects of lower nestling weight on survival to the autumn or next breeding season (e.g. Gustafsson & Sutherland 1988, Smith et al. 1989, Tinbergen & Boerlijst 1990, Lindén et al. 1992) while this has not been evident in others (e.g. Nur 1984, Lessels 1986, Boyce & Perrins 1987, Dijkstra et al. 1990). Possible reasons for the contrasting results might be differences in study species, population densities, sample sizes and the degree of manipulation. Furthermore, lower nestling weight has been observed to affect the breeding performance of manipulated offspring negatively (Gustafsson & Sutherland 1988, Lindén 1990). In the present study, no obvious effect of manipulation on breeding success of female offspring was found. However, the lower weight of males from enlarged litters might reflect lower reproductive success in competition for females. For instance, in two species of *Microtus* voles, the males with highest reproductive success had on average higher body weight than other males in the population (Sheridan & Tamarin 1988, Nelson 1994).

The results from the present study indicate, that the weaning weight seems to be important for long-term survival of females, while it has no obvious effect on the survival of males. The reason for this difference between sexes might be linked to the fact that males were heavier than females at weaning, but further studies are needed to clarify survival differences between sexes. As an interesting result the weaning weight of female offspring seemed to explain their future survival quite independently of the litter manipulation group. It seems that from every treatment group the heaviest female offspring - that also were the "strongest" - had the best chances of survival (Fig. 3). It is possible that non-surviving female offspring were in poorer condition because of genetic background or maternal effects. In the present study the number of non-surviving offspring was not related to initial litter sizes (Mappes et al. 1995, this study), indicating that large litter *per se* does not constrain the survival of pups. Further, the weight of the mother, the only measure of the mother's phenotypic quality available, did not correlate with the proportion of surviving offspring.

Why larger female offspring then survive better than smaller ones quite independently of litter manipulation? According to our recent results, the size

at birth in particular indicates the phenotypic quality in the bank vole, both in the laboratory and in the field. It seems that the weight at birth correlates significantly with the weight at weaning, and in females also with the age at maturation and the size of their first litter (Mappes & Koskela unpublished data). Unfortunately, in the present experiment the offspring were not marked individually before weaning and so it is not possible to analyse the importance of birth weight from the current data set. However, these results may indicate that even though the litter enlargements caused lower weights of female offspring, this did not change the originally underlying effect of birth weight (or other brood traits, Tinbergen & Boerlijst 1990) on their subsequent performance. If this was true, it could indicate that optimal reproductive effort in small mammals is mainly determined during pregnancy, whereas in (altricial) birds the time when parents feed their young is more important determinant of optimal brood size.

To conclude, bank vole mothers nursing litter sizes different from their original do not seem to gain any fitness benefits in terms of number of surviving offspring until breeding. The study demonstrates the long-term influence of litter size manipulation on size of individuals which may, at least in males, affect future reproductive success in the bank vole. The results are consistent with the majority of experiments published in birds, finding that costs of enlarged litter appears rather as offspring trade-offs than as parent trade-offs. However, as the present study concerns only one mammal species and was conducted during one season, care must be taken when interpreting the results for mammals in general. Obviously more experimental studies are needed for a better understanding of reproductive trade-offs in mammals.

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Table 1. The survival of offspring from weaning to breeding season in relation to litter manipulation group, sex and weaning weight. Sample sizes are in brackets. The survival of female and male offspring was further studied by linear logistic regression-models with variables weaning weight and the interaction weight by treatment.

	Reduced	Control	Enlarged
Females	66.7 (15)	63.2 (19)	50.0 (20)
Males	35.0 (20)	60.0 (25)	53.8 (26)
Both sexes	48.6 (35)	61.4 (44)	52.2 (46)
Logistic regression models			
	χ^2	df	p-value
Females			
Weight * Treatment	1.34	2	0.513
Weight	8.11	1	0.004
Males			
Weight * Treatment	2.47	2	0.291
Weight	0.78	1	0.377

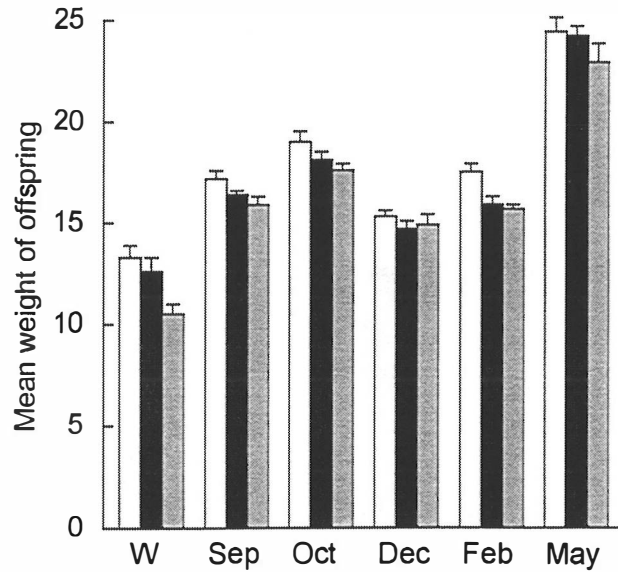


Fig. 1. Mean weight of offspring (mean \pm SE in grams) among the treatments from weaning to the beginning of breeding season. W, weaning (August, 30 days old); Sep, September; Oct, October; Dec, December; Feb, February; May (time of first reproduction). Open bars, reduced litters; solid bars, control; shaded bars, reduced litters.

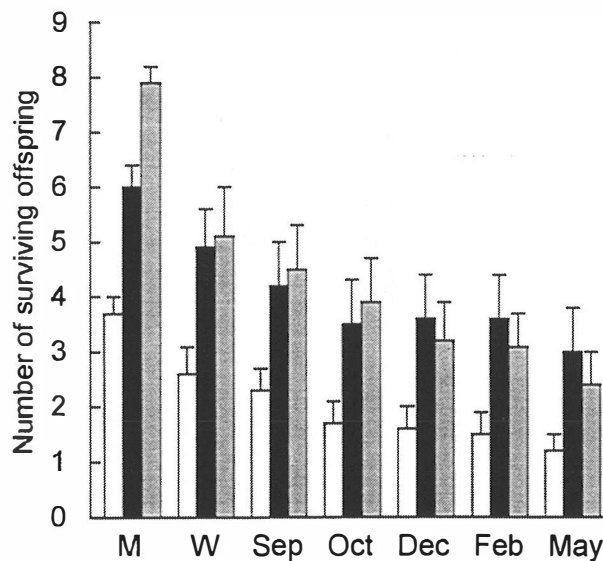
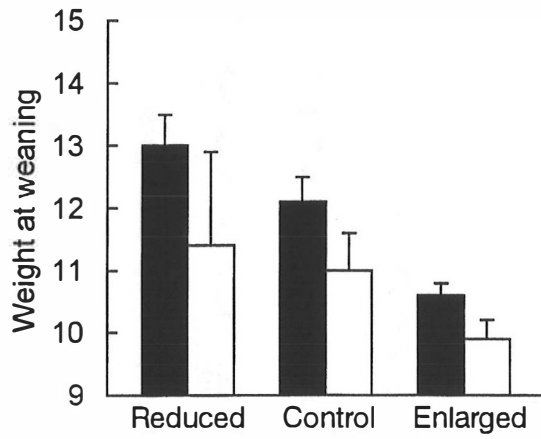


Fig. 2. Mean number of offspring among the litters during the study. M, time of manipulation (offspring 1-2 days old). For other details see legend for fig. 1.

A)



B)

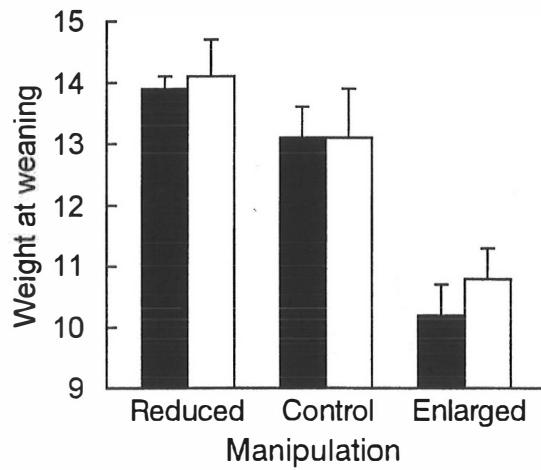


Fig. 3. Survival of female (A) and male (B) offspring over winter in relation to weight at weaning (mean \pm SE in grams) in different treatments. Solid bars, survived individuals; open bars, died individuals.

V

Genetic basis of the trade-off between offspring number and quality

By

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GENETIC BASIS OF THE TRADE-OFF BETWEEN OFFSPRING NUMBER AND QUALITY

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Summary. One of the main tenets of the modern life-history theory is the trade-off between the number and quality of produced offspring (Roff 1992, Stearns 1992). The theory predicts negative genetic correlation between these traits since both are closely related to fitness of individuals (Rose 1985, Curtsinger et al. 1994). However, this genetic basis for the trade-off has been tested only to a limited extent (Roff 1992, 1996). Despite a lack of genetic evidence, the trade-off between the size and number of offspring forms already the basis for the most evolutionary models predicting optimal reproductive allocation in vertebrates (e.g. Charnov and Downhower 1995, Charnov 1997). Here we report a significant negative phenotypic correlation between the number and size of offspring at birth both in laboratory and field populations of the bank vole *Clethrionomys glareolus*. Further, experimental results demonstrate that larger size at birth increases subsequent breeding success of female offspring. Finally, negative genetic correlation between the number and size of offspring confirms that the trade-off is under genetic control. Our study gives novel evidence for antagonistic pleiotropy, which may greatly affect the rate and direction of evolution of the two related life-historical traits (Slatkin and Frank 1990, Curtsinger et al. 1994).

A commonly observed determinant of offspring size is the negative relationship between number and quality of offspring (first formulated by Smith and Fretwell 1974). According to this trade-off, large size at birth should increase the quality of offspring through faster growth, faster maturation process or better survival, when simultaneously the number of offspring and hence the overall fecundity is energetically or mechanically constrained. This has been experimentally verified in viviparous reptiles (Sinervo and Licht 1991a,b, Sinervo et al. 1992): when the clutch sizes were hormonally enlarged during the pregnancy, the size of offspring at birth (and subsequent quality) decreased. There are corresponding correlative evidence from mammals (Roff 1992), but in these studies the trade-offs during the nursing could not be separated from the prenatal trade-offs. Furthermore, there is no evidence for the positive relationship between the size at birth and quality of offspring in wild mammals. However, recent epidemiological studies in humans indicate that decreased size at birth increase the risk of the development of later diseases, e.g. ischaemic heart disease (Barker et al. 1989).

Evidence for the first assumption of the trade-off between number and quality of offspring, a negative phenotypic correlation between the two traits,

has been observed in many vertebrates (reptiles and small mammals, but not commonly in birds, reviewed in Roff 1992). This applies also with the present findings in the bank vole, where the litter size correlates negatively with mean body mass and head width of offspring at birth both in the laboratory and in the field (Table 1). We studied the relationship between size and quality first by determining the age at maturation of female offspring in the laboratory. To be able to study the size-number trade-off separately during pregnancy and nursing, the litter sizes of mothers were manipulated after parturitions (see methods). The results show that the size of female bank voles at birth explains their subsequent breeding success: the larger the size at birth the earlier the age at maturation (Fig. 1). Litter size enlargements decreased and reductions increased the size of offspring at weaning in relation to the control litters (Fig. 2a). However, size at weaning (20 days) did not explain the age at maturation in females (Fig. 2b). This indicates that the trade-off between number and size of offspring is relatively more important during the pregnancy than during the lactation. In further analysis the age at first reproduction did not correlate with litter size ($r = -0.12$, $n = 145$, $P = 0.15$). This result does not support the idea, that later maturing females would benefit from better fecundity.

We further studied whether the size at birth predicts the breeding success of bank vole females also in natural conditions. The results of the field experiment suggest that the size at birth significantly explains the probability of breeding and the size of the first litter (Fig 3a,b). Body mass at weaning (20 days) or just before the experiment (50 days) did not affect the probability to breed ($G = 0.08$, $n = 29$, $P = 0.772$; $G = 1.99$, $n = 29$, $P = 0.159$, respectively), neither did body mass correlate with litter size ($r_s = 0.15$, $n = 26$, $P = 0.46$; $r_s = 0.23$, $n = 26$, $P = 0.26$, respectively). This emphasises further the relative importance of size at birth for the breeding success of females.

Mother's size (e.g. size of reproductive tract) is hypothesized to be an important mechanical factor determining the trade-off between number and size of neonates in mammals (Maynard Smith et al. 1985). We analysed the whole breeding data of laboratory colony of female bank voles and we did not find any clear relationship between the size of mother and litter size or total mass of litter (head width of mother vs litter size : $r = 0.02$, $n = 153$, $P = 0.794$; vs. litter mass: $r = 0.07$, $n = 150$, $P = 0.367$). This may indicate that some other factors (e.g. energetic) are more important determinants of the trade-off than pure mechanical factors.

Trade-offs between traits mould the evolution of life-history strategies. However, it has been argued that this reasoning requires information about the genetic basis and covariance of the traits in question (e.g. Reznick 1985, Pease and Bull 1988). According to our knowledge, the negative genetic correlation between the number and size of offspring has been reported only once (in threespined stickleback, Snyder 1991). We estimated the heritabilities of the number and the size of offspring at birth in the bank vole (Table 1). The calculated heritabilities are rather high as compared to those reported for life-history traits earlier (Mosseau and Roff 1987), indicating high amount of genetic variance in these traits. Furthermore, in addition to a negative phenotypic correlation between the number and size of offspring there is also a negative

genetic correlation between the traits (Table 1). This negative relationship has been theoretically predicted to occur when both traits are closely related to fitness (Rose 1982). In addition, the observed antagonistic pleiotropy can, at least partly, explain the high genetic variation in the related traits (Curtisinger et al. 1994).

Our study gives novel evidence for the evolutionary trade-off between litter size and offspring quality in mammals. The age at first reproduction in females seems to be determined by their size at birth. In general, breeding at as early age as possible should be beneficial in an increasing population (Caswell 1982). These conditions are typical in small rodent populations, where densities usually increase through the breeding season. There may also exist a negative relationship between the age at maturation and growth that would lead to later maturation and larger adult size (Lande 1982). However, this trade-off is not supported in the bank vole, because females maturing later do not have higher fecundity (e.g. larger litters).

Our results indicate that the trade-off between size and number of offspring is more important determinant of optimal reproductive effort during pregnancy than during lactation. This is further supported by earlier litter size manipulations in the field (Mappes et al. 1995b, Koskela 1998, Mappes and Koskela, unpublished data), where the number of offspring during nursing did not clearly affect the quality of offspring. This is contradictory to the results from bird studies, in which nursing time is critical for optimal reproductive allocation (Gustafsson and Sutherland 1988, Roff 1992).

Future studies should focus on studying the optimal allocation between the number and quality of offspring which maximizes fitness in female mammals. Litter size manipulations should be performed during pregnancy to be able to experimentally manipulate offspring size at birth. Consequently, it would be possible to determine the optimal energetic investment in each offspring (Charnov et al. 1995; Sikes 1998) and the possible ecological mechanisms (e.g. via survival) and genotype-environment interactions, which might be important in the evolution of reproductive effort in female mammals.

METHODS

Study species. The bank vole *Clethrionomys glareolus* is a common rodent in Europe. In central Finland (62°37' N, 26°20' E) female bank voles reproduce from early May to September during that time giving birth up to four litters. Only females from the first cohort mature during the summer of their birth (Mappes et al. 1995a). A great proportion of females reproduce only during one breeding season (P. Jonsson, E. Koskela and T. Mappes, unpublished). Breeding bank vole females are strictly territorial and possessing a territory is a prerequisite for breeding. Particularly when resources are scarce and population density is high, there is competition between individuals for the possibility to start breeding. Thus, age at first reproduction is an important fitness component in bank vole females (Bujalska 1988).

Phenotypic correlations. The field data consists of females ($n = 104$) trapped from the wild population in central Finland during breeding seasons 1995-97. Females were trapped just before parturitions and brought to the laboratory to give birth. The laboratory colony females ($n = 184$) were maintained under 18L:6D photoperiod in standard mouse cages with wood shavings and hay as bedding. Food (laboratory rodent chow) and water were available *ad libitum*. Laboratory females were from first to third generation descendants of wild individuals. After parturitions the number and size (body mass and head width) of pups were determined. Mothers were weighed and measured for head width, which is a substitute of their structural size (T. Mappes and E. Koskela, unpublished).

Breeding success experiments. From the laboratory colony 84 mothers (out of 184) were selected to the breeding success experiment in laboratory. Their pups were weighed and their head widths measured at birth. Pups were cross-fostered randomly between the mothers for randomising maternal effects during nursing. To study the trade-offs separately during pregnancy and nursing, litter size manipulations were performed. Litters were assigned to three treatment groups (reduced: -2 pups; enlarged: +2 pups, control: original litter size). Offspring were weaned from their foster mothers at the age of 20 days. Female offspring ($n = 149$) were mated repeatedly from the age of 40 days until their first parturition (maturation age).

56 female offspring were selected from the control litters to the field experiment. At the age of 50 days they were released simultaneously and evenly to eight outdoor enclosures (á 0.25 ha, density 28 females/ha) at June. Description of habitat and enclosures is found in Mappes et al. (1995b) and Koskela et al. (1997). After a habituation period of one week, three mature males were introduced to each enclosure. Before parturitions, all remaining females ($n = 29$) were removed from enclosures to standard breeding cages in the laboratory where they gave birth.

Quantitative genetic analyses. The individuals used in quantitative genetic analyses were first to third generation descendants of wild individuals originating from the population in central Finland (see number of individuals in Table 1). All the analyses were performed in the laboratory. Heritabilities and genetic correlations were calculated for litter size and mean offspring size at birth (body mass and head width). Heritabilities were estimated by regressing daughter values to the values of mothers and genetic correlations were ~~calculated from mother-daughter~~ covariances (Falconer and Mackay 1996, Lynch and Walsh 1997, Roff 1997). Standard errors of genetic correlations were estimated by using the formula of Reeve (1955) and Robertson (1959). All daughters were randomly cross-fostered between the mothers in order to eliminate or, at least, decrease phenotypic covariance between the mothers and their daughters (maternal effects during nursing).

Data analyses. The effects of body size at birth on the age at maturation were analysed by regression analyses (Fig. 1). To verify the results of regression

analyses the hierarchical analysis of variance was performed, where fosters were within litters and litters within mothers. The effect of body mass or head width (covariates) were entered to the model after the effects of mothers, litters and fosters. In the models both body mass ($F = 4.03$, $P = 0.048$) and head width ($F = 10.37$, $P = 0.002$) significantly explain the age at first breeding. We also controlled for the effect of same mother and enclosures on the probability of breeding and litter size in the field experiment (Fig. 2). In the analyses where only one female per mother ($n = 18$) where used the body mass explained significantly the probability of breeding ($G = 5.36$, $P = 0.021$) and litter size ($r_s = 0.58$, $P = 0.011$). Enclosure does not affect the probability of breeding ($G = 11.2$, $df = 7$, $P = 0.130$) or litter size ($F = 1.96$, $df = 7$, $P = 0.122$).

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Table 1. Heritability estimates (h^2) for litter size, mean body mass and head width of offspring at birth and phenotypic (r) and genetic correlation (r_A) between these traits. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. n_1 = number of mother-daughter pairs, n_2 = number of mothers.

	$h^2 \pm \text{S.E.} (n_1)$	$r (n_2)$	$r_A \pm \text{S.E.} (n_1)$
Litter size	$0.68 \pm 0.22 (89)$ **		
Head width	$0.51 \pm 0.21 (73)$ *		
Body mass	$0.77 \pm 0.21 (78)$ ***		
Litter size vs. head width	in laboratory in field	-0.47 (102) *** -0.40 (164) ***	$-0.30 \pm 0.27 (73)$
Litter size vs. body mass	in laboratory in field	-0.47 (104) *** -0.53 (184) ***	$-0.54 \pm 0.16 (78)$

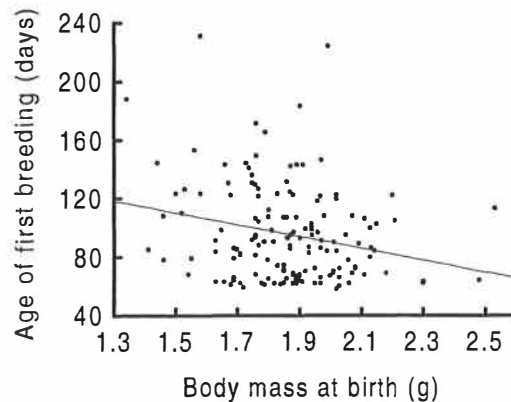


Fig 1. Larger size at birth decreases maturation age in female bank voles. Both the body mass (regression: $\text{age} = -46.11 \pm 14.34 (\text{S.E.}) * \text{mass} + 186.24 \pm 26.87 (\text{S.E.})$, $F = 10.34$, $n = 149$, $P = 0.002$) and the head width at birth (regression: $\text{age} = -27.79 \pm 8.36 (\text{S.E.}) * \text{head width} + 329.67 \pm 70.22 (\text{S.E.})$, $F = 11.06$, $n = 149$, $P = 0.001$) significantly explains the age at first breeding.

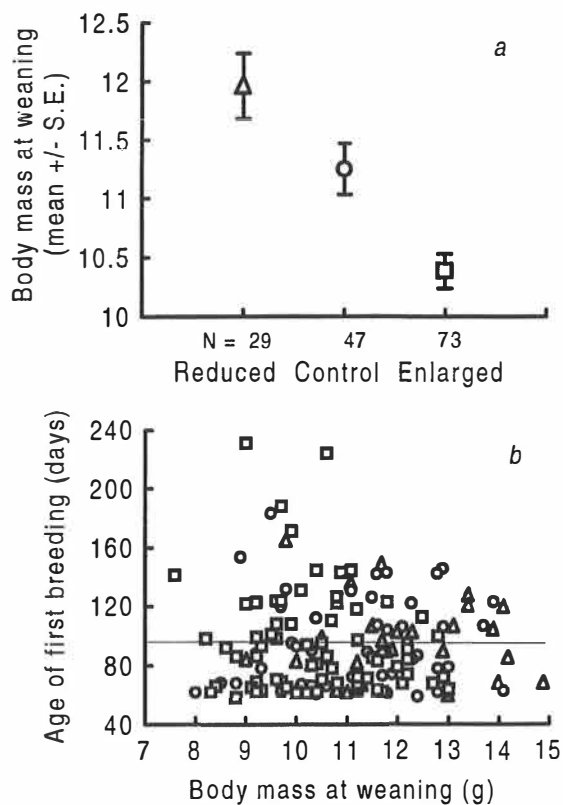


Fig. 2. Litter size manipulation affects the body mass at weaning (a) (One-way ANOVA: $F = 15.06$, $P = 0.000$), but the higher size at weaning does not enable earlier maturation in female bank voles (b) (Analysis of covariance: treatment: $F = 0.57$, $P = 0.568$; body mass at weaning: $F = 0.23$, $P = 0.634$, interaction: $F = 0.56$, $P = 0.571$).

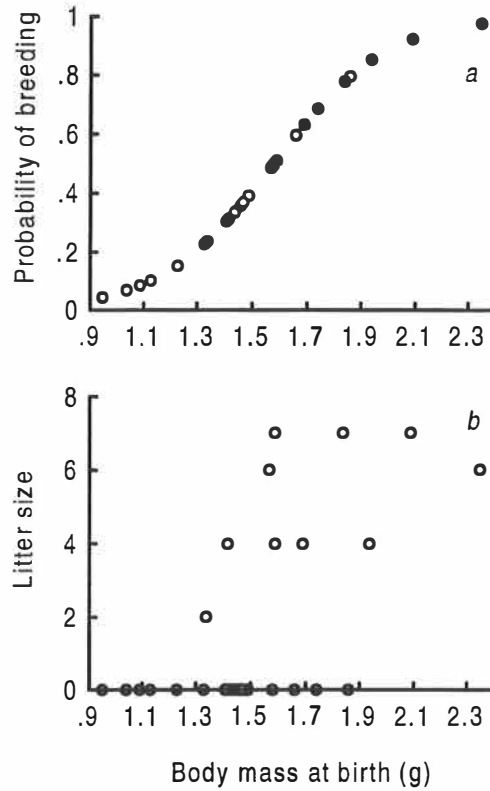


Fig. 3. Breeding success of bank vole females in relation to their body mass at birth in the field experiment (a). Probability is predicted by logistic regression model: $\text{logit } \pi(\text{body mass}) = 4.87 \pm 2.11 \text{ (S.E.)} * \text{body mass} - 7.70 \pm 3.28 \text{ (S.E.)}$. The effect of body mass is significant ($G = 8.85, P = 0.003$). Filled circles represent females which bred ($n = 13$) and unfilled circles those who did not breed ($n = 16$) during the experiment. Body mass at birth also correlates positively with litter size (b) ($r_s = 0.57, n = 26, P = 0.003$).