

Tuula Oksanen

Cost of Reproduction and
Offspring Quality in the Evolution of
Reproductive Effort

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ABSTRACT

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Any organism has a limited amount of resources, which have to be partitioned among several activities such as maintenance, growth and reproduction. Reproductive effort is defined as the proportion of parental resources devoted to fecundity and offspring survival. The extent of reproductive effort is restricted by trade-offs that cancel out the advantages of increasing investment in reproduction. This thesis presents an investigation into the determinants of reproductive effort in the bank vole, *Clethrionomys glareolus*, focusing mainly on two major life history trade-offs i.e. cost of reproduction and trade-off between offspring number and quality. Insights into other potentially important processes such as maternal effects, density dependent selection and differential allocation of maternal effort are included. The analyses are based on data acquired by several different experimental approaches. Hormonal manipulation of litter size revealed that costs of reproduction are even stronger determinants of reproductive effort than previously recognised. The trade-off between offspring number and quality, however, was not as unambiguous as expected. Simultaneous manipulations of offspring number and size suggested that the effects of offspring number on the future prospects of the offspring were indirect and mediated through offspring body size at birth and the quality of maternal care. The benefits of large body size at birth were dependent on the rearing environment whereas the disadvantages of small body size were related to survival probability and costs of reproduction. The reproductive decisions of females were independent of the population density and the quality of their mate. The results highlight the importance of adopting new aspects in even the most extensively studied topics such as the evolution of life histories.

Key words: *Clethrionomys glareolus*; cost of reproduction; life history trade-offs; litter size manipulation; offspring quality; reproductive effort.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-V. I am the main writer in all papers and I have performed large part of the work in each study.

- I Oksanen, T. A., Jonsson, P., Koskela, E. & Mappes, T. 2001. Optimal allocation of reproductive effort: manipulation of offspring number and size in the bank vole. *Proc. R. Soc. Lond. B* 268: 661-666.
- II Oksanen, T. A., Jokinen, I., Koskela, E., Mappes, T. & Vilpas, H. 2002. Manipulation of offspring number and size: benefits of large body size at birth depend upon the rearing environment. Manuscript (submitted).
- III Oksanen, T. A., Koskela, E. & Mappes, T. 2002. Hormonal manipulation of offspring number: maternal effort and reproductive costs. *Evolution* 56: 1530-1537.
- IV Oksanen, T. A., Koivula, M., Koskela, E. & Mappes, T. 2002. Survival cost of reproduction evident in small females and in high densities. Manuscript.
- V Oksanen, T. A., Alatalo, R. V., Horne, T. J., Koskela, E., Mappes, J. & Mappes, T. 1999. Maternal effort and male quality in the bank vole, *Clethrionomys glareolus*. *Proc. R. Soc. Lond. B* 266: 1495-1499.

1 INTRODUCTION

1.1 Reproductive effort

Reproductive effort is defined as the proportion of parental resources devoted to fecundity and offspring survival. Any organism has a limited amount of resources at its disposal, and these have to be partitioned between reproductive and non-reproductive activities. High reproductive effort at any age leads to a better reproductive performance at that age, but reduces the reproductive contribution in succeeding stages of the life history. Natural selection favours individuals, who maximise their genetic contribution to future generations. This contribution will generally be greatest for those individuals producing the greatest number of offspring that survive and reproduce (Williams 1966, Gadgil & Bossert 1970, Hirshfield & Tinkle 1975).

Reproductive effort is most frequently measured as the number of offspring produced, but also other methods such as the total mass of the offspring, offspring mass relative to the body mass of the mother, food intake and daily energy expenditure have also been used. Experimental studies in various study species suggest that individuals adjust their current reproductive effort according to ecological factors (e.g. Julliard et al. 1997, Both 1998), their own state and ability to raise the young (e.g. Lack 1947, Pettifor et al. 1988) and the quality (Wedell 1996, Kolm 2001) and attractiveness of their mate (de Lope & Møller 1993, Petrie & Williams 1993). The extent of reproductive effort is ultimately restricted by trade-offs that cancel out the advantages of high investment in reproduction (Stearns 1989).

1.2 Cost of reproduction

Life history theory predicts a trade-off between current reproductive effort and subsequent reproduction and/or survival. This trade-off has been termed the

cost of reproduction hypothesis (Williams 1966, Stearns 1976, Bell 1980, Partridge & Harvey 1985). Costs of reproduction are separated into survival costs, where current reproduction decreases an individual's probability of future survival, and fecundity costs, where current reproductive effort affects future reproductive capacity. Reproductive effort during the current reproductive event is therefore a compromise between the benefits of offspring production and the associated costs (Lindén & Møller 1989). Costs of reproduction, as viewed by theories of life history evolution (i.e. physiological costs), arise from the allocation of limiting resources among growth, maintenance and reproduction. All costs of reproduction such as ecological costs involving interactions with the external environment, however, may not be included in this category (Reznick 1992).

Several different approaches are applicable in measuring the costs of reproduction. Phenotypic and genetic correlations between life history traits may be estimated from observational data, whereas manipulation of life history traits or environments and measuring the responses to selection require an experimental approach (Lindén & Møller 1989, Stearns 1989, Reznick 1992, Rose & Bradley 1998, Sinervo & Svensson 1998). Costs of reproduction have been rarely revealed by observational studies (Bryant 1979, Ekman & Askemo 1986), as uncontrolled underlying factors cause positive phenotypic correlations between the traits (Partridge & Harvey 1985). Experimental manipulations of reproductive effort are therefore the most frequently utilised method of estimating the costs of reproduction. A number of brood size manipulation studies carried out in birds suggest that fecundity costs are more frequently found than survival costs (see Lindén & Møller 1989 for review). Brood size manipulation studies, however, have been criticised for including only the costs of rearing the chicks, while other phases of reproduction i.e. egg production and incubation may include costs as well (Monaghan & Nager 1997). Recently, several studies have shown that egg production and incubation do include costs in terms of offspring rearing capacity (Heaney & Monaghan 1996, Monaghan et al. 1998, Cicho 2000, Ilmonen et al. 2002). Studies examining the effects of production costs on the future survival and fecundity, however, are still rare (Visser & Lessels 2001).

In mammals, costs of gestation are generally slight compared to those of lactation (Clutton-Brock 1989), during which the food intake and daily energy expenditure of females increases considerably (Millar 1978, Kenagy et al. 1990, Sikes 1995, Künkele 2000). Observational studies have often failed to reveal survival or fecundity costs of reproduction (Murie & Dobson 1987, Millar et al. 1992, but see Huber et al. 1999), and even the results from experimental studies are inconsistent (Hare & Murie 1992, Koskela 1998, Neuhaus 2000). In mammals, offspring survival during nursing is dependent on the mother's survival. Therefore, it has been suggested that, selection should favour females who can keep survival costs of reproduction constant over small changes in their reproductive effort, and that this may have hindered their detection (Tuomi 1990). Another group of animals where the costs of reproduction have been widely studied is lizards, in which novel hormonal and surgical

manipulation techniques have been applied to reveal both survival (Sinervo & DeNardo 1996, Miles et al. 2000) and fecundity costs (Landwer 1994) of reproduction.

As evidence for costs of reproduction is abundant, and their existence is one of the cornerstones of life history theory, it has been suggested that rather than trying to prove that costs exist, studies should concentrate on how ecology and physiology translate into the demographic costs that ultimately constrain reproduction (Sinervo & Svensson 1998). The physiological aspects of life history trade-offs, and especially the role of the endocrine system in mediating the trade-offs, have opened a new perspective in this widely studied topic of life history theory (Ketterson & Nolan 1992, 1999, Finch & Rose 1995, Rose & Bradley 1998, Zera & Harshman 2001).

1.3 Trade-off between offspring number and quality

Another important life history trade-off determining optimal reproductive effort is the trade-off between offspring number and quality. Offspring quality is an important component of fitness, as an individual's genetic contribution to future generations is not dependent on the number of offspring produced but the number of offspring attaining reproductive age. Life-history theory predicts that as the number of offspring produced increases their quality decreases, because the amount of resources, that parents can allocate to each individual offspring (i.e. parental investment) decreases (Smith & Fretwell 1974). The hypothesis is based on the early work of Lack (1947), who suggested that a bird would lay the number of eggs that resulted in the maximum number of fledged young.

It has since been found that the most common clutch size in unmanipulated avian populations is often less than that which gives rise to the most fledglings. Numerous studies have tested the hypothesis by experimentally enlarging brood size, and confirmed that birds can rear more young than their average clutch size (Dijkstra et al. 1990, Godfray et al. 1991, Vander Werf 1992, Monaghan & Nager 1997). In the long-term, clutch size enlargement has been found to have a negative effect on the survival of the offspring (Smith et al. 1989), and it is currently widely recognized that simply counting the number of young fledged in a single breeding attempt does not represent an adequate measure of parental fitness (Roff 1992, Stearns 1992). Offspring number and quality studies in mammals have followed the same pattern suggesting that in free-living populations the most productive litter size is not as common as expected (Morris 1986, Boutin et al. 1988). Experimental studies, however, have revealed that litter size enlargement often has a negative effect on offspring body mass at weaning (Koskela 1998, Koskela et al. 1999, Humphries & Boutin 2000), but long-term effects of weaning size have not been adequately tested.

As experimental increase in offspring number frequently leads to a decrease in body size, and similar negative relations have also been found on body size already at birth from unmanipulated populations (Kaufman & Kaufman 1987, Smith et al. 1989, Sinervo & Licht 1991a), the focus of number and quality studies has lately shifted from studying the effects of offspring number towards the significance of body size and early development (Desai & Hales 1997, Lindström 1999, Sinervo 1999, Metcalfe & Monaghan 2001, Lummaa & Clutton-Brock 2002). This approach has benefited from the development of new manipulation methods that enable altering offspring body size during the early stages of their development (Sinervo & Licht 1991b, Williams 2001). Moreover, the foci of experimental studies have been expanded from searching the optimum of trait values to mapping the sources of the apparent phenotypic variation in life history traits (Sinervo et al. 2000).

1.4 Aims of the study

The aim of this thesis was to study the evolution of reproductive effort in the bank vole through several different approaches, and to adopt some new aspects in this intensively studied field of life history evolution. The thesis therefore presents a thorough investigation into the roles of two major life history trade-offs i.e. the cost of reproduction and the trade-off between offspring number and quality, on the evolution of reproductive effort with insights into potentially important processes such as maternal effects, density dependent selection and differential allocation of maternal effort.

2 METHODS

2.1 Keeping and breeding bank voles

The study species, the bank vole (*Clethrionomys glareolus* Schreber 1780), is a common mammalian species in northern Europe (Stenseth 1985). The main habitats are forests and fields, and the diet consists of forbs, shoots, seeds, berries and fungi (Hansson 1985). Home ranges of males are overlapping and larger than those of females which do not overlap (Bondrup-Nielsen & Karlsson 1985). The density of breeding females is limited through their territoriality (Bujalska 1985), and in high densities the maturation of young females is suppressed by social interaction among females (Kruczek and Marchlewska-Koj 1986, Marchlewska-Koj 1997). The patterns and amplitude of density variation show considerable geographical variability, and both stable and cyclic populations are found (Hansson & Henttonen 1985). In our study area, females give birth to a maximum of four litters during the breeding season, which lasts from late April to September. The litter size ranges from two to ten offspring (Koivula et al. 2002). Nursing period lasts around twenty days, after which the pups reach total independence from their mother. Females do not distinguish their own pups from foreign ones which enables manipulating and cross-fostering litters when the pups are young (Mappes et al. 1995). Bank voles have good trappability, and they are not sensitive to disturbance, which allows monitoring populations by live-trapping. The individuals used in the experiments were either wild caught bank voles or laboratory born descendants of wild individuals originally captured at the study site. All animals were housed in the laboratory for at least one month before starting the experiments.

The laboratory populations of bank voles used in the studies were maintained in the Experimental Animal Unit of the University of Jyväskylä. Laboratory facilities of Konnevesi Research Station were used to observe the birth of the offspring and to collect blood and parasite samples during the field experiments. Adult animals were housed individually in standard mouse cages measuring 43 Δ 26 Δ 15 cm. Wood shavings and hay were used as bedding, and

food pellets and water were available continuously. Litters were separated from their mothers at the age of 20 days, and housed together until the age of 40 days, after which they were separated in different cages. The animals were maintained on a 16 L : 8 D photoperiod. All individuals were individually marked either by ear tags or by toe clipping immediately after birth.

Studies I - IV were conducted mainly in 11 outdoor enclosures (0.2 ha each) situated in a field at our study site in Konnevesi, central Finland (62°37'N, 26°20'E). To monitor individuals, 20 multiple-capture live traps were distributed in each enclosure in a 5 × 4 grid with 10 m separating each trap. Each trap was covered with a galvanized-sheet-metal chimney that reduced exposure to precipitation and temperature extremes. The enclosures were emptied of all voles and other small rodents by a thorough live-trapping effort before releasing experimental animals. While in the enclosures, the animals were dependent on naturally occurring food resources, except during the trapping periods when the traps were baited with oats, sunflower seeds and a slice of potato. Enclosure fences were constructed of a 1.25 m high, galvanized sheet metal embedded 0.5 m into the ground. The fences were high enough to enclose the study populations, but did not necessarily prevent possible entry of predators (e.g. red fox (*Vulpes vulpes*), least weasel (*Mustela nivalis nivalis*), stoat (*Mustela erminea*) or avian predators), particularly in winter when the snow cover was high.

Experimental litters were produced by pairing mature males and females in the laboratory. The pair was placed in the same cage for a week. Pregnant females were checked once or twice a day for parturition. Immediately after birth was detected, the mother and the offspring were measured, and the offspring were sexed and individually marked. Newborn pups were weighed with electronic scales and their head width was measured under a microscope. Adult individuals were weighed with electronic scales as well, and their head width was measured with a digital calliper. After measurements, the pups were returned to the breeding cage with their mother.

2.2 Manipulations of reproductive effort and environmental characteristics

2.2.1 Offspring number and size

Offspring number and size manipulations were performed within two days after birth of the offspring to study the effects of different offspring number and size combinations on the future performance of the mothers and offspring (Fig. 1). Litter size enlargement was performed by adding two extra pups to each litter. The additional offspring originated from litters of the same size as the target litter. Manipulation of offspring body size was performed by replacing the litter with bigger pups originating from litters that were smaller in number

by 2 to 3 pups. The manipulations were combined to form a 2×2 factorial design, consisting of four different manipulation groups: 1) original offspring number and size, 2) original offspring number, but heavier pups, 3) original offspring size plus two pups and 4) heavier offspring plus two pups. Simultaneously with the offspring number and size manipulation, all pups in all litters were replaced with pups originating from several different donor mothers. The method enabled randomising the genetic and maternal effects of biological mothers on the experimental litters.

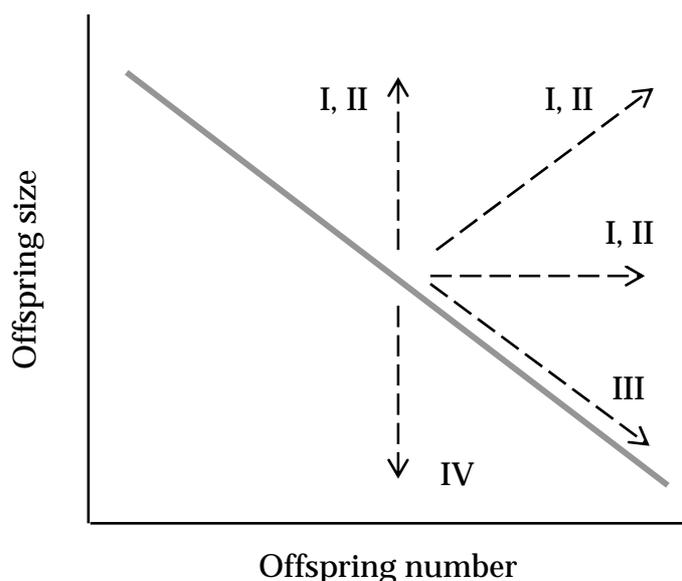


FIGURE 1 Graphical presentation of the directions in which the manipulations (dashed lines) expanded the natural variation in offspring number and mean body size at birth (solid line). Numbers I - IV refer to original papers.

After manipulation, mothers and pups were transferred to the enclosures in their breeding cages. The cages of four mothers (one from each manipulation group) were placed near the corners of each enclosure, and left open so that the mothers could move the pups into the enclosure. The subsequent success of the offspring was followed up by live-trapping. In experiment I, the design was used to study a mothers' allocation of reproductive effort between offspring number and size by assessing the effects of different allocation tactics on the long-term reproductive success of mothers. In experiment II, the same experimental design was applied to study the relative significance of body size at birth and rearing environment (i.e. litter size) on the short-term future performance of individual offspring.

2.2.2 Hormonal manipulation method

A hormonal manipulation method was developed for manipulating litter size during gestation. Females were injected with exogenous gonadotropin hormones prior to pairing them with males. Gonadotropin from human

menopausal urine (hMG, Sigma Chemical Co., St. Louis, MO, USA) was used to generate greater follicular ovulation than normal and human chorionic gonadotropin (hCG, Sigma Chemical Co., St. Louis, MO, USA) was used to induce ovulation. Females receiving this treatment produced larger litters of smaller sized individual offspring than normal. The change in offspring body mass at birth, as a function of increasing litter size, was comparable to the trade-off between these traits observed in natural variation.

In experiment III, manipulated litters were used to study the effects of enlarged litter size on the future prospects of the offspring and the mothers (Fig. 1). One control mother and one experimental mother were placed in each enclosure with their litters. The growth, survival and reproduction of individuals was monitored by live-trapping after weaning in the following winter. In experiment IV, offspring from experimental litters were used to study the effects of body size independently from litter size and the quality of the mother (Fig. 1). Offspring born to control and hormone treated mothers were cross-fostered within two days after birth to foster females, whose litter sizes were not changed. Foster females nursed their litters in the laboratory, and after weaning the offspring were placed in the enclosures in varying densities. One half of the individuals in each enclosure originated from litters of hormone treated mothers and the other half from litters of the control mothers. Maturation, reproductive success and survival of young females was monitored by weekly trapping periods.

2.2.3 Breeding density

Breeding bank vole females are territorial, and the availability of free territories restricts their reproduction (Bujalska 1985). Breeding density therefore has the potential for influencing the fitness of an individual through its effects on the probability of breeding. Moreover, population density during the nursing period has been found to affect the weaning success of females (Koskela et al. 1999). In experiment IV, the manipulation of breeding density was combined with the manipulation of body size at birth, to study whether the effects of body size at birth on fitness characteristics of females are dependent on environmental factors. The density was defined as the number of females in the enclosure. 92 females were divided among 11 enclosures in varying densities (5–13 females per enclosure). In addition to the females, 84 males were released one week later (4–12 males per enclosure) to balance the population. The proportion of males and females in each enclosure was adjusted as close to 50:50 as possible, the total number of animals in each enclosure thus being 9 – 25.

2.3 Measures of offspring quality

2.3.1 Growth, maturation and survival

Body mass and head width were used as measures of body size. Body mass was measured with electronic scales to the nearest 0.01 g in infants and 0.1 g in adults and juveniles, and it represented the nutritional component of body size, whereas head width measured under a microscope or with a digital calliper represented the skeletal body size of individuals. Offspring body size was measured at birth, after the nursing period (20 days of age) and at least once later (i.e. 45 days of age, end of the breeding season, winter or beginning of the next breeding season) depending on the experimental design. A residual from the regression of body mass on head width was used as a measure of condition in experiment III. In experiment II, proportional gain in body size was calculated to estimate the body mass and head width gained during the independent life in relation to the size that was acquired during the nursing period: $(\text{size at 45 days} - \text{size at 25 days}) / \text{size at 25 days}$.

The maturation of young females was followed up in experiment IV by intensive live-trapping that continued every week throughout the breeding season. Females that were found to be pregnant with their first litter were transferred to the laboratory, where the birth of the litter was observed. Maturation age was determined as the date of parturition. After measurements, the females and offspring were returned into enclosures. In experiment I, the size of the first litter of female offspring was observed in the laboratory, and used as a measure of offspring quality.

The survival of the offspring was determined by live-trapping. As the offspring are totally dependent on their mother for the first 15 days of their lives, the probability of survival was estimated separately for the nursing period and the subsequent period of independent life. The length of the latter period varied from three to ten months depending on the study design.

2.3.2 Physiological condition

In addition to body size and survival, some physiological measures of offspring condition were used to determine individual quality. Haematological measures included haematocrit, white blood cell count (WBC) and offspring immune function (serum immunoglobulin G). Two retro-orbital blood samples were collected from each individual at the age of 45 days in heparinised capillary tubes (I.D. 0.5 – 0.6 mm). One of the capillary tubes was used for white blood cell count in Brüker haemocytometer chamber (cells/ml). The other capillary tube was centrifuged at 12 000 g for 5 min in a haematocrit centrifuge (Heraeus Biofuge) to separate the serum from the blood cells. Haematocrit was expressed as the percentage proportion of packed red blood cells in respect to total volume of blood. The plasma was stored at -20°C and used to determine the

concentration of cerum IgG (U/ml). To measure specific immune response, each individual was immunised with an injection of bovine gamma globulin (BGG, Sigma Chemical Co. St. Louis, MO, USA), and the response was measured from another blood sample that was collected four weeks later. Plasma levels of total IgG and anti-BGG specific antibodies were determined by micro plate enzyme-linked immunosorbent assay (ELISA).

The intensity of infection by *Eimeria* spp., genera of an intestinal protozoan (coccidian), was determined from faecal samples that were collected at the age of 45 days by placing the animals individually in small plastic containers for half an hour. The faeces were stored in 2.5% aqueous potassium dichromate ($K_2Cr_2O_7$) to make 1 ml of suspension. The suspension was centrifugated for 3 min at 1750 rpm and the pellet was resuspended in a saturated magnesium-sulphate ($MgSO_4$) flotation solution. The intensity of parasite infection was estimated by counting the number of oocysts in a counting chamber, and the count was transformed into the number of *Eimeria* spp. oocysts per gram of the original sample.

2.4 Measures of adult performance

2.4.1 Reproductive effort

In experiment III, reproductive (RE) effort was measured as litter mass relative to mother's body mass:

$$RE = L * M_O / M_m,$$

where L is the number of offspring alive per litter (litter size) at birth or at weaning; M_O is mean offspring body mass at birth or at weaning and M_m is the postpartum body mass of the mother. The estimate of RE at birth represented the effort during pregnancy, whereas the estimate of RE at weaning represented the effort invested in nursing the offspring. The change in body mass during the nursing period was measured to find out if the females responded to the increased energy demand during lactation by increasing their food consumption or by depleting body reserves. Reproductive success of the females was measured in experiments I and III as the number and size of offspring alive at weaning, during the following winter and at the beginning of the next breeding season.

In experiment V, maternal effort in relation to the quality of their mate was measured as the number and size of their offspring and by their food consumption and milk production during the nursing period. Food consumption during lactation was measured as the difference in food pellet weight between the first and fifteenth day of lactation, and expressed as in grams per day. Milk production was measured when the pups were 10 days

old. The pups were separated from the mother for 3 h, after which the mothers were allowed to nurse the pups for 2 h. The pups were weighed before and after the nursing to determine the amount of milk they had received. Two measures of milk production were used: total amount of milk produced (sum of milk yields in a litter) and milk produced per pup (average yield per pup).

2.4.2 Future survival and fecundity

The future fecundity of females was studied in outdoor enclosures. After the birth of the experimental litters in the laboratory, the females were paired again in postpartum oestrus. The pairing was observed in a behavioural arena to ensure that all females had mated and thus had an equal opportunity to produce a second litter. After nursing and weaning the experimental litter in the enclosures, the females were trapped to check whether they were pregnant with a second litter. Being pregnant while lactating pups is a natural state for females in the wild (Bronson 1989). The future survival of females was determined by live-trappings at the end of the experiment.

2.4.3 Male mating success

The quality of the males was determined by mating success trials in which each male competed against three to eight other males in a behavioural arena. A male pair and a female in oestrus were released in to an arena (1m Δ 1m), and the behaviour of the animals was observed until one of the males mated with the female. The quality of the male was determined as the proportion of opponents against which he successfully copulated with the female. The estimates were corrected for inequality in the quality of the opponents with an equation:

$$Q = F n_F / \Psi n_F + (1 - S) n_S \beta$$

where Q is the corrected quality estimate; F is the mean quality value of the unsuccessful opponents; n_F is the number of unsuccessful opponents; S is the mean quality value of successful opponents and n_S is the number of successful opponents. After mating success trials, the males were paired with randomly chosen females to estimate the reproductive effort of the females in relation to the quality of their mate. The heritability of mating success was estimated from a father-son regression as twice the slope of the regression line (Roff 1997).

3 RESULTS AND DISCUSSION

3.1 Determinants of reproductive effort

3.1.1 Cost of reproduction

Cost of reproduction (Williams 1966) turned out to be an even more important determinant of optimal reproductive effort than previously recognised (Lindén & Møller 1989, Tuomi 1990, Mappes et al. 1995). In experiment III, females nursing hormonally enlarged litters had lower survival through the nursing period compared to control females. Also the probability of producing a second litter was lower in experimental females, but only if their RE at weaning was high, meaning that they had successfully nursed the first litter. The cost of reproduction in terms of future fecundity, therefore, seemed to be a joint effect of high RE at birth and at weaning. The negative impact of current reproductive investment on future survival has previously been shown in lizards (Sinervo & DeNardo 1996, Miles et al. 2000). Litter size enlargement experiments in mammals, however, have often failed to find survival or fecundity costs of reproduction (Hare & Murie 1992, Mappes et al. 1995, Humphries & Boutin 2000, Neuhaus 2000), and the prevailing conception has been that mothers do not trade-off their own condition for the quality of their offspring. It is suggested that simple litter size enlargement experiments do not capture the full scope of endocrine-triggered costs associated with the ovarian regulation of large litters. The hormonal manipulation method, however, enabled the range of normal variation in litter size to be expanded, and the significance of reproductive costs in the evolution of optimal reproductive effort was revealed.

In experiment IV, the survival cost of reproduction was shown to be dependent on a mother's body mass at birth and population density. Reproduction entailed survival costs especially for small females and for females reproducing at high densities. The functions of body mass and density, however, were separate suggesting that the costs were generated by two different mechanisms. Cost of reproduction was therefore an important

determinant of reproductive effort for both the mothers and the offspring. The optimal reproductive effort for the mothers would have been to produce offspring that are large enough to bear the costs of reproduction. The offspring themselves, on the other hand, should assess their reproductive decisions according to their own condition together with the quality of the environment. Support for the cost of reproduction hypothesis has also recently been found in an extensive experiment carried out in natural bank vole populations in eight independent areas (Koivula et al. 2002).

3.1.2 Offspring number and quality

The reproductive success of females in relation to litter size (control/enlarged) and body size (control/large) of their offspring was highest in females nursing enlarged litters. The number of offspring alive in the beginning of the next breeding season was highest in enlarged litters, and there was no difference in body size of the offspring. Offspring size manipulation did not have any effect on offspring body size or number at the beginning of the next breeding season. Also the litter size of female offspring did not differ between the treatments, suggesting that offspring quality was the same in all groups. Offspring body size at weaning was negatively affected by litter size enlargement suggesting that the amount of reproductive effort the mother invested per pup during nursing period decreased when litter size was enlarged. The effect of the manipulation on body size, however, was not permanent, as the pups grew to the same size as offspring in other groups after reaching independence from their mother. Therefore, it is likely that the decreased body size at weaning was caused by restrictions in the ability of the mothers to support enlarged litters, and that it did not impair the quality of the offspring.

The finding that an enlarged litter size does not decrease offspring quality is quite the opposite of previous studies where this trade-off has been found (e.g. Gustaffson & Sutherland 1988, Dijkstra et al. 1990), and it is possible that due to yearly and seasonal changes in environmental conditions, our results may not represent the only possible outcome (see e.g. Sinervo & DeNardo 1996, Sinervo 1999). It may also be argued that a trade-off between offspring number and quality was not found because females adjust their reproductive effort according to the demands of future reproduction (Williams 1966), and thereby the effort put into a single reproductive attempt may be increased, whereas in the long run the amount of investment that results in the highest lifetime reproductive success is restricted by costs of reproduction. It is concluded that, during evolution, the selective pressure against larger offspring size has been stronger than that against larger litter size, as the mothers were unable to rear larger pups more successfully, but enlarged litter size did not decrease the number or quality of offspring surviving to reproductive age. Thus, offspring size may be a more evolutionarily fixed trait than litter size, and females appear to be able to increase the number of offspring reaching the reproductive age by producing larger litters, whereas increasing offspring size benefits neither the mother nor the offspring.

In experiment III, the number of offspring alive per litter in the following winter was compared with the reproductive effort of control and gonadotropin treated females. The mean RE of the control mothers was closer to the most productive value of RE at birth than that of experimental females nursing enlarged litters. The probability of complete litter loss, however, did not differ between the treatments. The results therefore suggest that the quality of the offspring was lower in enlarged litters than in control litters, which is contradictory to the interpretation of results from experiment I. It seemed, however, that in addition to the effects of litter size, offspring performance in large litters was affected by the ability of the mother to support the litter. The increase in the mothers' body mass during the experiment was negatively related to RE at weaning in the experimental group, but not in control females. This suggests that when RE at birth was increased by the hormonal manipulation, high investment in nursing the litter became costly in terms of decreased gain in body mass, whereas control females were able to respond to the increased energy demand during nursing irrespective of the effort they invested in nursing the litter. The low survival rate of offspring in enlarged litters was therefore not caused exclusively by the trade-off between offspring number and quality.

3.1.3 Breeding density

Breeding density had a significant effect on the manifestation of reproductive costs. Females reproducing in high-density enclosures had lower probability to survive to the end of the breeding season than females reproducing in low-density enclosures. It is therefore suggested that females should adjust their reproductive effort according to their environment, and that the evolution of optimal reproductive effort is modified by the temporal variation in environmental quality. This conclusion is supported by earlier studies showing that life history traits in voles are sensitive to changes in their immediate environment (Ostfeld & Canham 1995, Ergon et al. 2001).

3.1.4 Male quality

Male quality did not have any significant effect on the litter size, mean offspring body size at birth, amount of food the females consumed or the quantity of milk they produced. On the contrary, offspring body size was related to their mother's body mass and the amount of food consumed correlated positively with litter size. Offspring body size at the age of 20 days was positively related to the amount of food consumed and the total amount of milk produced. Male quality, however, affected neither the characteristics of the litter nor the extent of maternal effort measured as food consumption and milk production. Females mating with high quality males, therefore, did not gain any direct benefits, and they did not adjust their maternal effort according to the quality of their mates. However, as male mating success proved to be a highly heritable trait, females

gained indirect genetic benefits (male offspring with high mating success) from mating with a high quality male.

The results are contradictory to the prediction of the differential allocation hypothesis, which suggests that females should invest more in reproduction with high quality males, if the quality of the mate affects her reproductive success (Burley 1988). It is suggested that bank vole females did not adjust the extent of their maternal effort according to male quality, because the benefits of adjustment would not have been large enough to exceed the costs. The differential allocation hypothesis has gained support from several experimental studies (e.g. de Lope & Møller 1993, Petrie & Williams 1993, Gil et al. 1999, Cunningham & Russell 2000, Kolm 2001), while contradictory results are scarce (Sanz 2001, Smiseth et al. 2001). Despite the convincing evidence, testing the hypothesis has invoked also criticism and discussion (Møller & de Lope 1995, Witte 1995, Sheldon 2000, 2001, Birkhead et al. 2000, Colegrave 2001, Cunningham & Russell 2001, Gil & Graves 2001, Petrie et al. 2001).

The hypothesis has been tested either by manipulating the appearance of the mate or by experimentally allocating mates of different attractiveness and then recording the reproductive output of the individuals that received the treatment. The latter approach that was used in experiment V has been proposed to have two weaknesses (Sheldon 2000). First, it is suggested that the character that is being responded to may not be determined correctly. In experiment V the quality of the mate was defined as his mating success. Therefore, there was no need to define the exact character assuming that all the females responded to the same trait. Second, it is suggested that excluding the possibility that the effect represents genetic differences between the sires may be impossible. In experiment V, females did not adjust their maternal effort according to the quality of their mate. The heritability estimate of male mating success was, however, substantially high suggesting that females gained genetic benefits from mating with high quality males. It was therefore suggested that females confined themselves to these genetic benefits and did not increase their effort during the nursing period.

3.2 Determinants of offspring quality

3.2.1 Litter size

In experiment I, where litter size was manipulated by adding offspring after the birth of the pups, negative effects of enlarged litter size on the quality of individual offspring were not found, except for the smaller body size at weaning. The survival probability of offspring reared in enlarged litters even tended to be better than the survival probability of offspring reared in control litters. When the body size was manipulated during pregnancy in experiment III, however, the survival probability of offspring reared in enlarged litters was

significantly lower than that of control offspring. Hormonal litter size enlargement also had a negative effect on mean offspring body size at weaning, but the effect disappeared by winter. Offspring condition estimated from standardised residuals of head width on body mass was affected by the manipulation at birth, when offspring in enlarged litters had lower condition indices. The results therefore suggest that enlarged litter size had a negative effect on the quality of the offspring. The results are consistent with Williams (2001), who used anti-oestrogen tamoxifen to manipulate the number of eggs in zebra finch females (*Taeniopygia guttata*).

The results from experiments I and III are not fully comparable. In experiment I, the study design was factorial, and the effects of offspring number and size can be separated in the analysis, whereas in experiment III the negative effect of litter size and positive effect of body size at birth on survival probability were inseparable. Moreover, the manipulation methods in these experiments were different.

3.2.2 Body size at birth

The effects of body size at birth on the future performance of individuals were studied in relation to the size of the litter (I and III), the quality of rearing environment (II) and the density of breeding population (IV). In experiment I, offspring body size at birth showed substantial variation, but large body size did not seem to be an advantage or a weakness, as it did not affect the survival probability of individuals. It was concluded that selection against larger offspring size at birth has been substantial during evolution. If distribution of body sizes at birth in natural variation has been eroded by directional selection in past, in our study design, both offspring size manipulation groups may represent the flat top of the fitness curve. In that case, finding any substantial differences in survival probability between the manipulation groups would be unlikely.

In experiment II, the effects of body size at birth on the future performance of the offspring in the short-term were studied in relation to the quality of rearing environment (litter size). The study design was based on the prediction that as parents have to allocate their reproductive effort among their offspring, the amount of investment per individual offspring decreases with increasing number of offspring, and thus, the quality of the rearing environment is also expected to decrease (Lack 1947, Smith & Fretwell 1974). The effects of enlarged body size and rearing environment on the growth and immune function of young individuals strongly interacted. The growth of large individuals in poor environments was retarded in comparison to other groups. Moreover, there was an interaction between rearing environment and body size in the proportional gain in body size suggesting that offspring reared in poor environments were trying to compensate for their smaller body size at weaning after reaching independence from the mother. A similar trend was found in the specific response to immunisation with bovine gammaglobulin, whereas other measures of physiological condition were not affected by the manipulations. In

summary, according to this experiment, large body size at birth was an advantage in normal rearing environment, but a disadvantage in a poor one. The initially normal sized individuals reared in a poor environment were able to compensate for their small weaning size after reaching independence from the mother, whereas the large individuals in poor environments were not able to maintain their body size. The analyses provided new information about the mechanisms of body size related effects on offspring quality, and suggest that the benefits of large body size are dependent on the quality of the rearing environment.

In experiment III, the body size of offspring was manipulated in the opposite direction by a hormonal manipulation method. Offspring born to hormone treated mothers were smaller at birth and at weaning age. Moreover, body mass at birth was positively related to the probability of survival from birth to weaning suggesting that small body size entailed low survival probability. However, as the reproductive effort of the mothers was also manipulated, the effects of body mass on the performance of the offspring were likely to be mediated also through other circumstances during the nursing period such as the quality of maternal care.

Experiment IV was designed to study the effects of body size at birth independently from litter size and the quality of maternal care. Body size at birth did not affect the probability of breeding or the maturation age of females. It did however, have a significant positive effect on the survival probability of individuals. Moreover, the effect of body size at birth was interacting with the reproductive state of the females. The survival probability of breeding females was negatively affected by small body size at birth, whereas the survival prospects of non-breeding females were not affected by body mass at birth. Breeding was therefore costly for the small females in terms of survival. The results suggest that although the effects of body size are not always detected in the short term, they may emerge later in life (Metcalf & Monaghan 2001). Moreover, cost of reproduction (Williams 1966) seems to have the potential for modifying the evolution of life history traits.

3 CONCLUSIONS

The aim of this thesis was to study the importance of two life history trade-offs i.e. cost of reproduction and trade-off between offspring number and quality, in the evolution of reproductive effort. This question has puzzled evolutionary ecologists for decades, and lots of correlative and experimental data covering numerous study species and reproductive systems has been acquired. The results and conclusions, however, remain blurred by several confounding effects such as uncontrolled factors, weak study designs and the variety of study organisms. This project, therefore, embodied a challenge of finding new methods to overcome the drawbacks of earlier studies and achieving fresh perspectives into this old dilemma.

In this study, traditional offspring number manipulations were replaced with a simultaneous manipulation of offspring number and size, which enabled studying the fitness effects of different allocation tactics. Moreover, a new hormonal manipulation technique was utilized to preserve the natural trade-off between offspring number and body size at birth. Most of the studies were also designed to minimize the confounding effect of maternal quality by cross fostering the litters. The aim was not to study only the effects of litter size, offspring size or reproductive effort alone, but also to investigate how the evolution of these traits is modified by other potentially important factors such as correlated characters, selective environments and mate quality.

Cost of reproduction turned out to be an important determinant of optimal reproductive effort, although it has previously been suggested that mothers do not trade-off their own condition for the quality of their offspring (Lindén & Møller 1989, Tuomi 1990, Mappes et al. 1995). The severity of the costs in terms of future fecundity and survival was related to body size at birth, previous reproductive effort and density of the breeding population. The trade-off between offspring number and quality had an effect on the reproductive success of females as well. Offspring quality was more closely related to their body mass at birth than on the size of the litter in which they were raised. Negative effects of large litter size on the growth and survival of the offspring seemed to be stronger during the nursing period, when the offspring were

dependent on maternal care. This suggests that the effects of offspring number were, to some degree, mediated through the ability of the mother to support her litter. Body size at birth, in general, was positively related to the survival prospects of the offspring, but it was not commensurable to their quality. The benefits of large body size at birth were dependent on the quality of the rearing environment, suggesting that maternal environmental effects may also have the potential to influence the evolution of a mother's reproductive effort allocation between offspring number and size. Moreover, after reaching independence from their mother, offspring with an exceptionally small body size at birth seemed to survive the summer relatively well until they faced the costs of reproduction.

Females did not adjust their maternal effort according to the quality of their mates, even though they did gain genetic benefits through the high heritability of male mating success. The result was contradictory to the differential allocation hypothesis (Burley 1988), which suggests that females should invest more in reproduction with high quality males, if the quality of the mate affects her reproductive success. It was concluded that the benefits of adjustment might not have been large enough to exceed the costs. It was also found that the functions of body size at birth and breeding density were separated in the manifestation of survival costs of reproduction on young females. Adjusting litter characteristics in relation to environmental condition, did not therefore, seem to be beneficial for females.

The results provide new information about the evolution of reproductive effort in mammals. Previous studies have not been able to show that females may compromise their own survival for reproduction and the survival of their offspring. Therefore, the trade-off between offspring number and quality has been viewed as the main determinant of reproductive effort. The results obtained from the experiments included in this thesis, however, suggest that cost of reproduction has an important role in the evolution of reproductive effort as well. The findings highlight the importance of adopting new aspects in even the most extensively studied topics such as the evolution of life histories.

This work has benefited from integrating conclusions of studies with different experimental approaches. It includes the perspectives of mothers and offspring, two manipulation methods, different time scales and several angles on processes whose significance in the evolution of reproductive effort may be substantial. It seems evident that these approaches still have plenty to offer for life history research. In future, the focus of studies should shift towards investigating the roles of genetics and plasticity in the manifestation of maternal effects. Moreover, environmental aspects such as density and frequency-dependent selection should be integrated into the research of trade-offs to reveal the mechanisms that preserve variability in fitness related traits.

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YHTEENVETO

Lisääntymisen kustannukset ja poikasten laatu lisääntymispanostuksen evoluutiossa

Luonnonvalinta suosii yksilöitä, jotka pystyvät siirtämään mahdollisimman suuren osuuden geneettisestä perimästään tuleville sukupolville. Tässä onnistuvat parhaiten ne yksilöt, jotka tuottavat suurimman määrän lisääntymiskykyisiä jälkeläisiä. Kaikilla eliöillä on kuitenkin rajallinen määrä resursseja, jotka niiden on jaettava useiden toimintojen kuten oman kuntonsa ylläpitämisen, kasvun ja lisääntymisen kesken. Lisääntymispanostus on määritelty lisääntymiseen ja jälkeläisten elinkyvyn parantamiseen suunnattujen voimavarojen osuudeksi kaikista yksilön käytettävissä olevista resursseista. Lisääntymispanostuksen määrää rajoittavat allokaatiokustannukset, jotka kumoavat suuresta panostuksesta saatavan hyödyn.

Elinkiertoteorian mukaan allokaatiokustannuksia esiintyy mm. nykyisen ja tulevan lisääntymispanostuksen välillä. Suuri panostus nykyiseen lisääntymisyriytykseen aiheuttaa tuleviin lisääntymiskertoihin suunnatun panostuksen määrän pienenemisen. Nämä ns. lisääntymisen kustannukset ilmenevät joko heikentyneenä lisääntymismenestyksenä tai kasvaneena riskinä menehtyä ennen seuraavaa lisääntymiskertaa. Parhaaseen lopputulokseen johtava lisääntymispanostus on siis kompromissi saavutettujen hyötyjen ja kustannusten välillä. Allokaatiokustannuksia ilmenee myös jälkeläisten määrän ja laadun välillä. Kun kerralla tuotettavien jälkeläisten lukumäärä kasvaa, yksittäiseen poikaseen kohdistuvan panostuksen määrän oletetaan pienenevän ja poikasten elinkyvyn heikkenevän. Väitöskirjatyössäni olen selvittänyt näiden allokaatiokustannusten merkitystä metsämyyrän *Clethrionomys glareolus* lisääntymispanostuksen evoluutiossa. Lisäksi olen tutkinut koiraan laadun vaikutusta naaraan lisääntymispanostuksen määrään sekä kasvuympäristön ja populaatiotiheyden vaikutusta allokaatiokustannusten ilmenemiseen.

Metsämyyränaaraat synnyttävät kerralla yhdestä kymmeneen poikasta, joiden keskimääräinen syntymäkoko pienenee selvästi niiden lukumäärän kasvaessa. Naaraat voivat tuottaa jopa neljä poikuetta lisääntymiskauden aikana, ja uudet poikaset syntyvät tavallisesti heti edellisten lähdettyä pesästä noin 20 päivän ikäisinä. Tutkimuksessa lisääntymispanostusta manipuloitiin kahdella eri menetelmällä. Osatutkimuksissa I ja II poikasten määrää ja kokoa manipuloitiin niiden syntymän jälkeen korvaamalla alkuperäinen poikue joko samankokoisilla tai suuremmilla poikasilla sekä lisäämällä puoleen poikueista kaksi ylimääräistä poikasta. Osatutkimuksissa III ja IV käytettiin hormonaalista manipulointimenetelmää, jossa naaraille annettiin gonadotropiinihormonia injektioina ennen lisääntymistä. Hormonin vaikutuksesta poikasten lukumäärä kasvoi ja niiden syntymäkoko pieneni. Manipuloitujen poikueiden ja niitä hoitavien emojen menestystä tutkittiin Konneveden tutkimusasemalla sijaitsevilla aidatuissa ulkotarhoissa (á 0.2 ha). Koiraan laadun vaikutusta naaraiden lisääntymiseen tutkittiin myös Konneveden tutkimusasemalla sijaitsevilla aidatuilla ulkotarhoissa.

tymispanostukseen tutkittiin laboratorio-oloissa. Koiraideiden laadun mittana käytettiin niiden pariutumismenestystä, joka määritettiin käyttäytymiskokeen avulla. Kokeiden jälkeen kunkin koiraan annettiin lisääntyä yhden naaraan kanssa. Naaraiden lisääntymispanostuksen mittana käytettiin ruuan kulutusta, maidon tuottoa ja poikueen ominaisuuksia.

Tutkimustulokset osoittavat, että suuri lisääntymispanostus heikentää emojen todennäköisyyttä tuottaa toinen poikue ja selviytyä hengissä lisääntymiskauden loppuun. Lisääntymisen jälkeinen elinkyky heikkeni selvästi erityisesti syntymäkooltaan pienillä naarailla ja suurissa populaatiotiheyksissä. Lisääntymisen kustannukset ovat siis tärkeä lisääntymispanostuksen kasvua rajoittava tekijä, ja niiden merkitys lisääntymispanostuksen evoluutiossa lienee huomattava. Jälkeläisten lukumäärän vaikutus niiden laatuun ilmeni epäsuorasti syntymäkoon vaikutusten kautta. Poikuekoon kasvattaminen kokeellisesti saattoi vaikuttaa myös emojen poikasilleen antaman hoidon laatuun. Syntymäkoon vaikutus poikasten elinkykyyn oli suuri, mutta myös kasvuympäristöllä oli merkitystä. Suuri syntymäkoko oli edullinen kasvuympäristön säilyessä samana, mutta kun ympäristöä muutettiin epäedullisemmaksi kasvattamalla poikuekoko, suuresta koosta oli jopa haittaa. Pienen syntymäkoon haitat taas ilmenivät heikenneenä hengissäselviytymisen todennäköisyytenä sekä alttiutena lisääntymisen kustannuksille. Poikasten määrän ja syntymäkoon välillä havaittavalla negatiivisella korrelaatiolla näyttää siis olevan merkitystä myös jälkeläisten myöhemmän menestyksen kannalta. Koiraan laadulla ei ollut vaikutusta naaraiden lisääntymispanostuksen määrään.

Tutkimus paljasti uutta tietoa lisääntymispanostuksen evoluutiosta nisäkkäillä. Aikaisemmissa tutkimuksissa ei ole pystytty osoittamaan että naaraat vaarantaisivat oman elinkykynsä panostaessaan lisääntymiseen ja jälkeläistensä hengissä säilymiseen. Allokaatiokustannusta jälkeläisten määrän ja laadun välillä onkin pidetty tärkeimpänä lisääntymispanostuksen evoluutiota määräävänä tekijänä. Tässä tutkimuksessa kuitenkin havaittiin, että myös lisääntymisen kustannusten merkitys lisääntymispanostuksen evoluutiossa on merkittävä. Tutkimus poikkeaa aikaisemmista myös menetelmiensä osalta. Työssä käytettiin kahta erilaista manipulointimenetelmää, ja tuloksia tarkasteltiin sekä emon että jälkeläisten näkökulmista erilaisilla aikaväleillä. Lisäksi tutkimusasetelmiin yhdistettiin allokaatiokustannusten lisäksi myös muita lisääntymispanostuksen evoluution kannalta mahdollisesti tärkeitä tekijöitä kuten koiraan laatu, kasvuympäristö ja populaatiotiheys. Tulokset osoittavat, että uudet menetelmät ja näkökulmat avaavat uusia mahdollisuuksia jopa kaikkein laajimmin tutkittujen evoluutioekologian osa-alueiden kuten elinkiertojen evoluution tutkimukseen.

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