

# Maternal effort and male quality in the bank vole, *Clethrionomys glareolus*

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Parental investment in reproduction is adjusted according to potential benefits in terms of offspring survival and/or mating success. If male quality affects the reproductive success of a female, then females mating with high-quality males should invest more in reproduction. Although the subject has been of general interest, further experimental verification of the hypothesis is needed. We studied whether female bank voles (*Clethrionomys glareolus*) adjusted their maternal effort according to male quality, measured as mating success. To enable the measurement of maternal effort during nursing separately from male genetic effects the litters were cross-fostered. Further, the genetic background of male quality was examined. Male quality did not correlate with litter size or offspring size at birth. Offspring growth was positively related to food consumption and milk production of mothers. However, these direct measurements of maternal effort were independent of male quality. Male mating success appeared to be significantly heritable indicating that there are genetic benefits. Still, females did not adjust maternal effort according to the genetic quality of their offspring. We suggest that female bank voles gain significant genetic benefits from mating with high-quality males whereas they cannot improve their reproductive success by increasing maternal effort.

**Keywords:** heritability of mating success; life history; male quality; mammals; maternal effort; offspring quality

## 1. INTRODUCTION

Life-history theory predicts that the amount a female invests in reproduction will relate to the potential benefits she gains from the current reproductive attempt (Williams 1966; Gadgil & Bossert 1970). In conditions where a female can expect a relatively large fitness increment from reproduction, she should invest maximally in offspring production. Similarly, when the expected gain is low a female should defer reproduction or invest less until the net gain would be better.

If male quality affects the reproductive success (i.e. number and quality of offspring produced) of a female, then females mated to high-quality males should invest more in reproduction. There are several studies showing that females mated to preferred males produce more viable offspring (reviewed in Møller & Alatalo 1999). However, only a few studies indicate higher maternal effort in relation to male quality (see, for example, Burley 1988; de Lope & Møller 1993; Petrie & Williams 1993; Rintamäki *et al.* 1998).

Our study species, the bank vole (*Clethrionomys glareolus*), has a mating system in which males provide no material resources to the female or offspring. Females discriminate between males according to their social status and prefer dominant mates (Hoffmeyer 1982; Horne & Ylönen 1996). In this mating system it is possible to control for

several confounding effects that may confuse the relation between male quality and female reproductive effort. These effects may arise particularly in a situation where maternal care is dependent on paternal care (see, for example, Burley 1988; de Lope & Møller 1993) or because of an assortative mating pattern (see, for example, Rintamäki *et al.* 1998). Furthermore, if offspring inherit the high quality of their fathers (for example, a higher growth rate), this may lead to the erroneous interpretation that females increase their reproductive effort when mated to high-quality males.

The aim of this study was to examine whether male quality affected litter size and offspring size at birth or the maternal effort during nursing (milk production and food consumption). Further, the genetic background of male quality was examined. To enable the measurement of maternal effort separately from male genetic effects the offspring were cross-fostered between females.

## 2. MATERIAL AND METHODS

The animals used in the experiment were second-generation laboratory-born bank voles (*Clethrionomys glareolus*) originally captured in Konnevesi, central Finland (62°37' N, 26°20' E). The animals were housed in standard mouse cages measuring 43 cm × 26 cm × 15 cm and maintained in a 16 L : 8 D photoperiod. Wood shavings were used as bedding; water and food were continuously available. All the females whose maternal effort was measured had given birth to one litter before the

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experiment. Head width of all individuals was measured before the experiment and used as a factor illustrating parent size.

### (b) *Male quality trials*

Male quality was defined by a set of mating trials in which each male competed against at least three (range = 3–8, mean = 4.64, s.d. = 0.75) other randomly chosen males. A male pair and a female in oestrus were released in an arena (1 m × 1 m) with a Plexiglas cover. All females were injected supracollicularly with  $\beta$ -estradiol 3-benzoate (0.04 mg dissolved in 0.1 ml linseed oil) two days before the test to ensure their willingness to mate. The female's area consisted of one-half of the whole arena. The other half was divided between the two males. At the beginning of the test there was a wooden wall between the males and a wire mesh between the female and the males. The wire mesh enabled opposite sexes to detect each other.

During the first 10 min animals were allowed to familiarize themselves with the arena; then all the dividing walls were removed. Observations were made until ejaculation occurred. If no mating behaviour had been seen after 20 min the test was repeated at least 24 h later. The quality of males was determined as a proportion of opponents against which the male copulated successfully with the female. In these trials it was possible to test male mating success in a situation where both female choice and male–male competition were allowed to work.

Because the random selection of male pairs in male quality trials could have led to uneven distribution of opponent quality, these estimates were corrected with an equation,

$$Q = Fn_F / [Fn_F + (1 - S)n_S],$$

where  $Q$  is the corrected quality estimate of a male;  $F$  is the mean quality value of the opponents who failed to copulate;  $n_F$  is the number of the unsuccessful opponents;  $S$  is the mean quality value of the opponents who copulated successfully and  $n_S$  is the number of successful opponents (R. V. Alatalo, J. Höglund, A. Lundberg and P. Rintamäki, unpublished observations).

### (c) *Mating*

A new group of females (different from those used in male-quality trials) was paired with males of known quality. A randomly chosen male and a female were placed together in a cage for a week. These females were not manipulated with any hormones and the exact time of mating was dependent on their natural oestrus cycle, which is about four days. After a week the male and female were separated and the mass of females was followed up to detect the pregnancy. If mating did not turn out to be successful it was repeated after three weeks. After the first parturition the mother was mated again in postpartum oestrus with a randomly chosen male. This second mating was carried out because being pregnant while lactating pups is a natural state for females in the wild (Bronson 1989).

### (d) *Pup measurements and cross-fostering*

Pregnant females were checked twice a day to detect the parturition. Immediately after birth the pups were weighed and their head width was measured with a microscope. At the ages of ten and 20 days the pups were weighed again and their head width was measured with a dial caliper. All masses were measured with electronic scales. Litter means of offspring size were used in all the analyses. At birth the pups were also sexed and marked individually to identify them later. To randomize the genetic influence of parents on offspring growth, litters born

within two days of each other were cross-fostered. Each pup in a litter was replaced, resulting in a litter in which every pup came from a different mother. Litter sizes remained the same and sex ratios within litters were kept, where possible, the same as in the original litter. When the pups were 20 days old (weaning age) they were separated from the foster mother.

### (e) *Female food consumption and milk production*

Basal food consumption of individual females was defined before the experiment (when the females were not pregnant or lactating) by weighing their food pellets during one week. During lactation the food consumption was measured from parturition to the offspring age of 15 days. From this age the pups start to feed on pellets beside the mother. Female milk production was measured when the pups were 10 days old. The pups were separated from the foster mother for 3 h to let them consume the milk in their stomachs. After that the foster mother was 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 in the analyses: total amount of milk produced (sum of mass increases in a litter) and milk produced per pup (average mass increase of a pup in a litter).

### (f) *Heritability of male quality*

To find out whether male mating success had a genetic background, male quality trials were also performed (as described above) on male offspring after their maturation. The heritability of male mating success was estimated by regressing the quality value of one randomly chosen son against the value of his father. The heritability was estimated as twice the slope and the standard error was obtained by doubling the standard error of the regression (Roff 1997).

## 3. RESULTS

### (a) *Offspring size and number at birth*

Paternal quality had no significant effect on litter size or the mean offspring size at birth (figure 1*a,b*; table 1). Hence, paternal quality does not seem to have any positive impact on offspring number or size. Supported by the large sample sizes the effects were, if anything, negative. Offspring size was affected by maternal size, which had a positive effect on mean pup mass and head width (table 1). Paternal size did not explain offspring mass or head width (table 1). Male quality did not correlate with male size measured as head width ( $r = 0.17$ ,  $n = 85$ ,  $p = 0.111$ ).

### (b) *Maternal effort during nursing*

Female food consumption during lactation correlated significantly with basal food consumption ( $r = 0.54$ ,  $n = 43$ ,  $p < 0.001$ ) and the number of pups ( $r = 0.60$ ,  $n = 44$ ,  $p < 0.001$ ). The trade-off between litter size and mean mass of offspring in a litter at birth was also clear ( $r = -0.55$ ,  $n = 75$ ,  $p < 0.001$ ). Because of these dependencies, female basal food consumption and litter size were used as controlling factors in partial correlations ( $r_p$ ) below.

The amount of food females consumed during lactation did not correlate with the quality of their mates (controlling for basal food consumption and litter size:  $r_p = -0.09$ ,  $n = 39$ ,  $p = 0.578$ ). However, food consumption rate had a significant effect on mean offspring mass at the ages of ten

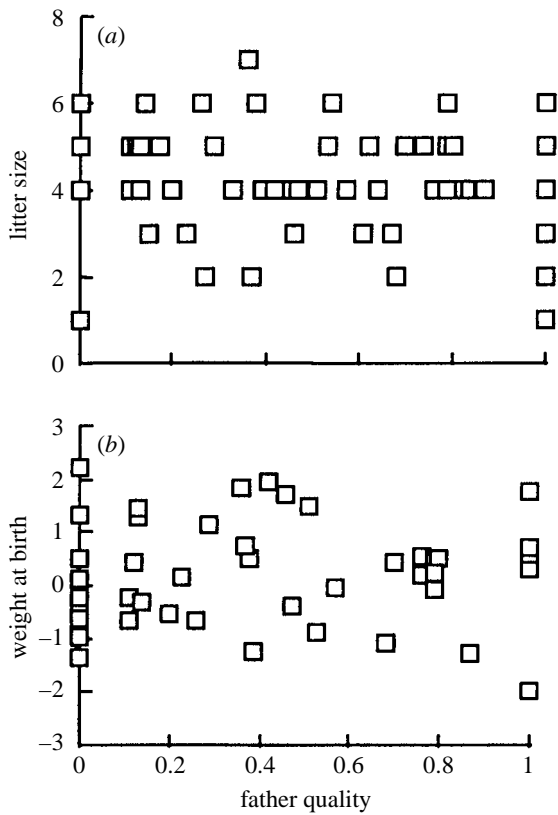


Figure 1. Paternal quality in relation to (a) litter size (Kendall's partial correlation controlling for maternal head width:  $r_K = -0.13$ ,  $n = 73$ ,  $p = 0.099$ ), and (b) offspring mass (in grams) at birth (multiple regression analysis  $b = -0.04$ ,  $t = -0.80$ ,  $n = 64$ ,  $p = 0.426$ ). Offspring mass has been standardized by litter size.

Table 1. Mean offspring size at birth in relation to male quality, the size of parents and litter size

(Multiple regression analysis;  $n = 64$  litters.)

independent variable	body mass (g)			head width (mm)		
	<i>b</i>	<i>t</i>	<i>p</i>	<i>b</i>	<i>t</i>	<i>p</i>
paternal quality	-0.043	-0.801	0.426	-0.159	-1.823	0.073
paternal head width	-0.006	-0.145	0.885	0.068	0.942	0.350
maternal head width	0.139	3.324	0.001	0.144	2.112	0.038
litter size	-0.099	-6.353	<0.001	-0.116	-4.563	<0.001
	$F_{4,59} = 12.354; p < 0.001$			$F_{4,59} = 6.336; p < 0.001$		

(controlling for basal food consumption and litter size:  $r_p = 0.34$ ,  $n = 39$ ,  $p = 0.028$ ) and 20 days (figure 2a). Offspring head width, on the contrary, was not affected by the amount of food eaten at the age of ten (controlling for basal food consumption and litter size:  $r_p = 0.16$ ,  $n = 39$ ,  $p = 0.325$ ) or 20 days (controlling for basal food consumption and litter size:  $r_p = 0.17$ ,  $n = 39$ ,  $p = 0.284$ ).

Neither the amount of milk produced per pup (controlling for litter size:  $r_p = 0.12$ ,  $n = 40$ ,  $p = 0.419$ ) nor the total

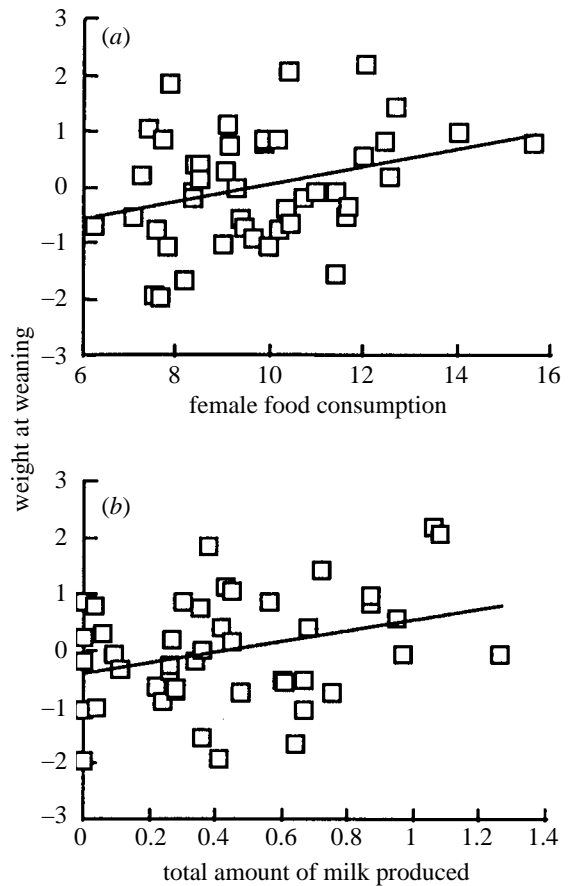


Figure 2. Mean offspring mass (in grams) at weaning in relation to (a) female food consumption (in grams per day) during the first 15 days of nursing (partial correlation controlling for litter size and female basal food consumption:  $r_p = 0.43$ ,  $n = 39$ ,  $p = 0.005$ ), and (b) the total amount of milk produced (in grams) in 2 h (partial correlation controlling for litter size:  $r_p = 0.34$ ,  $n = 44$ ,  $p = 0.020$ ). Offspring mass has been standardized by litter size.

amount of milk produced in 2 h (controlling for litter size:  $r_p = -0.002$ ,  $n = 40$ ,  $p = 0.989$ ) correlated with male quality. In addition, the amount of food the mother consumed while lactating had no effect on the total amount of milk (controlling for basal food consumption and litter size:  $r_p = -0.14$ ,  $n = 37$ ,  $p = 0.377$ ) or milk produced per pup (controlling for basal food consumption and litter size:  $r_p = -0.06$ ,  $n = 37$ ,  $p = 0.68$ ). Even so, the total amount of milk a female produced correlated significantly with mean offspring mass (figure 2b) and head width (controlling for litter size:  $r_p = 0.37$ ,  $n = 42$ ,  $p = 0.011$ ) at the weaning age. Hence, the more the female eats and the more milk she produces the bigger the pups are at the weaning age, but male quality does not affect the extent of these forms of maternal effort. The amount of milk received per pup and the total amount of milk produced (mean  $\pm$  s.d., in grams) were  $0.074 \pm 0.09$  ( $n = 47$  litters) and  $0.444 \pm 0.32$  ( $n = 47$  litters), respectively.

(c) Male quality and offspring growth

Mean litter size and foster-mother size affected offspring mass at the age of ten and 20 days (table 2). However, the quality of a male to which the foster mother was mated (i.e. the male whose offspring the foster

Table 2. *The relation between male quality and offspring size at the age of ten and 20 days*(Multiple regression analysis;  $n=42$  litters at ten days, 40 litters at 20 days.)

independent variable	mean body mass/g					
	10 days			20 days		
	<i>b</i>	<i>t</i>	<i>p</i>	<i>b</i>	<i>t</i>	<i>p</i>
male quality	-0.111	-0.330	0.743	-0.220	-0.473	0.639
male head width	0.025	0.101	0.920	0.005	0.017	0.986
foster head width	0.696	2.569	0.014	0.839	2.107	0.042
litter size	-0.242	-3.290	0.002	-0.314	-3.027	0.004
	$F_{4,37} = 4.103; p = 0.007$			$F_{4,35} = 3.537; p = 0.015$		

mother 'thought' she was nursing) did not have any effect on offspring mass (table 2). The same analysis applied to mean offspring head width at the age of 20 days gave parallel results (multiple regression, litter size:  $b = -0.08$ ,  $t = -4.88$ ,  $n = 40$ ,  $p < 0.001$ ; foster mother's head width:  $b = 0.17$ ,  $t = 2.48$ ,  $n = 40$ ,  $p = 0.018$ ; male quality:  $b = 0.10$ ,  $t = 1.32$ ,  $n = 40$ ,  $p = 0.193$ ).

#### (d) Genetic background of male quality

Father-one-son regression comparing the quality of fathers and sons revealed a significant value of heritability (figure 3). This indicates that male quality, measured as mating success, has a genetic background.

## 4. DISCUSSION

We used a novel approach to study the relation between maternal effort and male quality in the bank vole. In this animal's mating system, with uniparental care, it was possible to measure maternal effort separately from paternal effort and male genetic effects. The results do not support the hypothesis that females adjust their reproductive effort by investing more in the offspring of high-quality males.

Previously, adjustment of maternal effort has been found in insects where mate choice includes direct benefits to the female, such as courtship feeding (Gwynne 1984; Wedell 1996), or where there is postnatal paternal care in birds (Burley 1988; de Lope & Møller 1993). In the latter cases differences in the extent of maternal effort are, at least partly, caused by the variation in the amount of paternal effort (Witte 1995). In other words, it is difficult to know whether females increase their investment according to male quality or just compensate for decreased paternal effort (but see Møller & de Lope 1995).

If paternal care does not exist, all the variation in the amount of maternal effort can be interpreted as adjustment by females. Rintamäki *et al.* (1998) studied female investment in relation to male quality in lekking black grouse (*Tetrao tetrix*). In their study, females in good physical condition tended to mate with high-ranking

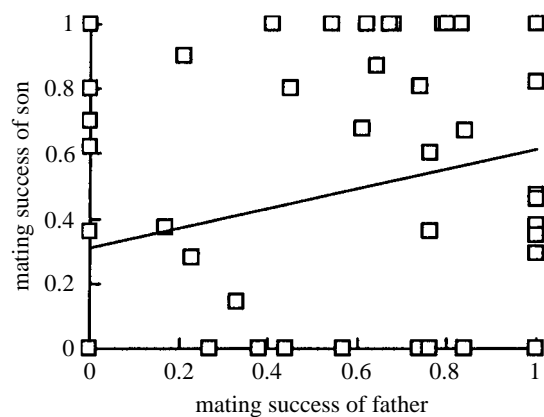


Figure 3. The heritability of mating success (father-one-son regression:  $h^2 \pm \text{s.e.} = 0.60 \pm 0.29$ ,  $n = 52$ ,  $p = 0.04$ ).

males and there was a positive correlation between male quality and clutch size. However, the authors suggest that an assortative mating pattern may have produced this relation and it could be wrongly interpreted as a male-quality effect. Petrie & Williams (1993) solved this problem by randomly pairing females with males of known quality. They found that peahens (*Pavo cristatus*) invested more in reproduction by laying more eggs when mated to peacocks with more elaborate trains. Contradictory to these earlier studies, no signs of increased effort during the prenatal period were found in the present experiment with considerable sample sizes. Nevertheless, if the adjustment of maternal effort exists in the bank vole, it should have been detected during nursing. Although offspring growth was positively related to the food consumption and milk production of mothers, these direct measurements of maternal effort were independent of male quality.

Whether females gain genetic benefits for their offspring from their mates has been widely studied in connection with the good-genes hypothesis of sexual selection theory (see, for example, Moore 1994; von Schantz *et al.* 1994; Alatalo *et al.* 1997; Sheldon *et al.* 1997). Overall, the recent meta-analysis of Møller & Alatalo (1999) indicates that male secondary sexual characters explain on average 1.5% of the viability of offspring, an effect comparable to low heritability estimates of fitness. Further, they found that the effects were stronger for studies where the target of sexual selection had been identified than for those with an unknown target of selection. This may explain the high heritabilities estimated for male mating success (present study) or dominance-related characters (Horne & Ylönen 1998) in the bank vole. Furthermore, the correlation between male quality and offspring viability might be significantly lower in this species.

Female bank voles did seem to gain increased reproductive success (male offspring with higher mating success) from mating with high-quality males. Nevertheless, they did not adjust the amount of maternal effort according to male quality, as life-history theory predicts. This may be because the benefits from adjusting maternal effort are not large enough to exceed the costs. This hypothesis is supported by our experiments, in which the

maternal effort of female bank voles has been studied by means of litter-size manipulations. For example, pups in reduced litters grew heavier but weaning mass did not affect the mating success of male offspring (T. A. Oksanen, R. V. Alatalo, E. Koskela, J. Mappes and T. Mappes, unpublished data) or reproductive success of female offspring (Koskela 1998). The lack of a positive relation between offspring size and quality may explain why females do not invest more in reproduction with high-quality males.

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