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Optimal allocation of reproductive effort: manipulation of offspring number and size in the bank vole

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The number of offspring attaining reproductive age is an important measure of an individual's fitness. However, reproductive success is generally constrained by a trade-off between offspring number and quality. We conducted a factorial experiment in order to study the effects of an artificial enlargement of offspring number and size on the reproductive success of female bank voles (*Clethrionomys glareolus*). We also studied the effects of the manipulations on growth, survival and reproductive success of the offspring. Potentially confounding effects of varying maternal quality were avoided by cross-fostering. Our results showed that the number of offspring alive in the next breeding season was higher in offspring number manipulation groups, despite their smaller body size at weaning. Offspring size manipulation had no effect on offspring growth or survival. Further, the first litter size of female offspring did not differ between treatments. In conclusion, females may be able to increase the number of offspring reaching reproductive age by producing larger litters, whereas increasing offspring size benefits neither the mother nor the offspring.

Keywords: life-history trade-offs; litter size manipulation; offspring size manipulation; offspring quality; reproductive success

1. INTRODUCTION

A phenotypic trade-off between the number of offspring produced and their size at birth is a common phenomenon in many species of birds (e.g. Smith *et al.* 1989), mammals (Kaufman & Kaufman 1987) and lizards (Sinervo & Licht 1991*a*). Clutch size manipulations in birds have long been the prevailing method of studying the consequences of this trade-off (Godfray *et al.* 1991). In most cases, experimentally enlarged brood (litter) sizes have not been found to increase the number of offspring surviving to independence (Pettifor 1993; Mappes *et al.* 1995; Koskela 1998), but rather to reduce it (Gustafsson & Sutherland 1988; Dijkstra *et al.* 1990). However, in some cases offspring quality seems to increase with enlarged clutch size (e.g. Robinson & Rotenberry 1991). These contradictory findings may partly arise from the fact that clutch size has been manipulated independently of offspring size.

There are several correlational studies that have reported a positive relation between egg size and subsequent growth and survival in birds (e.g. Nisbet 1973; Moss *et al.* 1981; Grant 1991). However, the results from experimental studies are quite different. In seed beetles (*Stator limbatus*), enlarged egg size has been shown to reduce the development time from egg to adult, but not to increase survival (Fox 1997). In birds, heavier eggs produce heavier offspring, but later in the breeding season offspring performance is more dependent on environmental factors and parental quality than on egg size (Amundsen & Stokland 1990; Bolton 1991; Magrath 1992; Blomqvist *et al.* 1997). Thus, the observed relationship between egg size and offspring survival is presumably caused by the confounding effects of parental quality. Based on these findings, it is evident that controlling for the effect of parental quality by cross-fostering is a necessity when studying the effect of offspring size on their future performance.

Variability in clutch size and variability in egg size have generated great interest as useful measures of individual fitness. Fitness is the sum of a large number of characters that can be broken down into two major components: the total number of offspring produced and the quality of these offspring. The number of reproducing offspring produced is a competent measure of individual fitness as it is more robust than the number of offspring born or weaned.

Only Sinervo (1990) and Sinervo & Licht (1991*a*,*b*) have previously studied the fitness effects of both offspring size and number simultaneously by hormonal manipulation and surgical methods in lizards (see the review in Sinervo 1999). We manipulated both traits in a fully factorial experiment using the bank vole (*Clethrionomys glareolus*) as a study species. Original litters were replaced with heavier pups from smaller litters and/or litter size was enlarged by two extra pups. Confounding effects of possibly varying maternal quality were excluded by cross-fostering all litters. We assessed the effects of offspring number and size as well as their interaction on offspring growth and survival from birth to the beginning of the next breeding season and on the reproductive success of female offspring. We also made inferences about the relative selection pressures on the number and size of

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individuals in a litter from nursing to the first breeding of the offspring.

2. METHODS

(a) *Study site and study species*

The study was conducted in Konnevesi, Central Finland (62°37′ N, 26°20′ E), using a laboratory and 11 0.2 ha outdoor enclosures situated in a fallow field. Two separate runs of the experiment were carried out: the first was in June–July (11 enclosures) and the second in July–August (nine enclosures) 1998. Winter survival of offspring from both runs was assessed in the enclosures from September 1998 to April 1999. The animals were housed in the laboratory in standard mouse cages with wood shavings as bedding and food pellets and water continuously available, whereas in the enclosures they were dependent on naturally occurring food resources. In order to monitor the animals, 20 multiple-capture live traps were distributed in each enclosure in a 5 × 4 grid with 10 m between traps. Each trap was covered with a galvanized sheet-metal chimney that reduced exposure to precipitation and temperature extremes. Enclosure fences were constructed of 1.25 m high galvanized sheet metal, which was embedded 0.5 m into the ground. The fences were high enough to enclose the study populations, but did not prevent possible entry of predators particularly in winter when the snow cover was high (e.g. red fox (*Vulpes vulpes*), least weasel (*Mustela nivalis nivalis*) and avian predators).

The study species, the bank vole (*C. glareolus*), gives birth to up to four litters during the breeding season, with the litter sizes ranging from two to eight offspring. The breeding season lasts from late April to September and pups reach independence before the age of three weeks. Bank voles have good trappability and they are relatively insensitive to disturbance. Females do not distinguish their own pups from foreign ones which enables litter manipulations and cross-fostering when the pups are young (Mappes *et al.* 1995). The individuals used in the experiment were second-generation, laboratory-born descendants of wild bank voles originally captured close to the study site. They had all reproduced once or twice earlier.

(b) *Offspring number and size manipulations*

The study began by pairing mature males and females in the laboratory. After birth pups were sexed, individually marked and their body size was measured. A microscope was used to measure head width to the nearest 0.1 mm and electronic scales to weigh body mass to the nearest 0.1 g. The litters were cross-fostered within two days of birth and offspring number and size manipulations were performed. We replaced all the pups in a litter, ending up with litters where every pup originated from a different mother.

A 2 × 2 factorial experiment was performed which consisted of two treatments: offspring number (±0 and +2 pups) and size manipulation (control and heavier pups). In the offspring number manipulation treatment a foster mother's original litter was replaced with pups from donor mothers who had the same litter sizes as the foster's initial litter size and two extra pups added. The offspring size manipulation was performed by replacing the original litter with an equal number of heavier pups from donor mothers whose litter sizes were two to three pups smaller than the initial litter size of the foster. This method was based on the fact that litter size and mean offspring body mass at birth are negatively correlated (in current data) ($r = -0.620$, $n = 171$ and $p < 0.001$) and, thus, offspring in small litters are heavier than offspring in large litters. A combination of these treatments gave us four manipulation groups: (i) original offspring number and size ($n = 17$ mothers), (ii) original offspring number, but heavier pups ($n = 17$ mothers), (iii) original offspring size plus two pups ($n = 16$ mothers), and (iv) heavier offspring plus two pups ($n = 17$ mothers) (figure 1*a*). Foster mothers' initial litter size (i.e. before manipulation) did not differ between manipulation groups (Pearson's $\chi^2 = 9.31$, d.f. = 12 and $p > 0.6$). In addition, their post-partum body mass did not differ between treatments (one-way ANOVA, $F_{3,28} = 0.828$ and $p > 0.4$).

Table 1. *Offspring body mass after manipulation, at weaning and in spring (next breeding season) in relation to offspring number and size manipulations*

(Nested ANOVA with foster mother (random effect) nested within offspring number and size manipulation and study run (fixed effects). MS, mean square.)

	d.f.	MS[a]	F	p
manipulation				
run	1	0.000	0.005	0.946
number	1	0.099	1.120	0.293
size	1	3.362	38.066	0.000
number × size	1	0.037	0.417	0.521
foster	69	0.092	3.942	0.000
error	434	0.023	—	—
weaning				
run	1	82.955	10.055	0.002
number	1	139.784	15.524	0.000
size	1	6.258	0.696	0.408
number × size	1	2.345	0.257	0.614
foster	48	12.550	7.871	0.000
error	205	1.594	—	—
spring				
run	1	0.177	1.561	0.223
number	1	0.254	2.231	0.147
size	1	0.108	0.952	0.338
number × size	1	0.187	1.641	0.211
foster	21	0.113	0.983	0.538
error	10	0.115	—	—

(c) *Offspring growth and survival*

After manipulations in the laboratory, four females (one from each manipulation group) were transferred to each enclosure in breeding cages. The cages were placed near the corners of the enclosures (one in each corner) under rainproof covers and left open so mothers could move the pups into the enclosure. This method has worked well in our previous studies (Mappes *et al.* 1995; Koskela *et al.* 1998, 1999). Pups were trapped at the age of weaning (25 days) in order to assess the number and size of pups alive. Offspring head width was measured to the nearest 0.1 mm with digital callipers and body mass to the nearest 0.1 g on electronic scales. The pups were released back into the enclosures after the measurements and the mothers were transferred to the laboratory. At the age of 45 days, the pups were trapped and removed to the laboratory. The second experimental run, which was identical to the first one but with different females, subsequently started in empty enclosures. After females in the second run had weaned their pups, offspring from both the first (*ca.* 75 days old) and second runs

Table 2. *Descriptive statistics (mean ± s.e.) for offspring head width (mm) after manipulation, at weaning and in spring (next breeding season) in relation to offspring number and size manipulations*

(Nested ANOVA with foster mother (random effect) nested within offspring number and size manipulation and study run (fixed effects). MS, mean squares.)

	d.f.	MS	*F*	*p*
manipulation				
run	1	1.570	4.678	0.034
number	1	0.018	0.055	0.815
size	1	10.253	30.925	0.000
number × size	1	0.014	0.045	0.832
foster	69	0.345	3.183	0.000
error	434	0.109	—	—
weaning				
run	1	2.593	6.484	0.014
number	1	6.331	14.509	0.000
size	1	0.563	1.293	0.261
number size	1	0.118	0.268	0.607
foster	48	0.608	7.833	0.000
error	205	0.077	—	—
spring				
run	1	0.128	1.010	0.325
number	1	0.108	0.877	0.358
size	1	0.039	0.318	0.578
number × size	1	0.059	0.480	0.495
sex	1	0.583	9.211	0.014
foster	21	0.134	2.114	0.124
error	9	0.063	—	—

Table 3. *Offspring head width (mm) after manipulation, at weaning and in spring (next breeding season) in relation to offspring number and size manipulations and sex*

number	original		enlarged	
size	original	large	original	large
manipulation	8.1 ± 0.1	8.4 ± 0.1	8.1 ± 0.1	8.4 ± 0.1
weaning	12.0 ± 0.1	11.9 ± 0.1	11.6 ± 0.1	11.5 ± 0.1
spring	13.8 ± 0.2	14.0 ± 0.1	13.7 ± 0.1	13.6 ± 0.1

(*ca.* 25 days old) were assigned to new enclosures to overwinter. Animals from different manipulation groups were evenly distributed into each enclosure. Offspring survival was assessed at the beginning of the next breeding season by trapping all individuals from the enclosures and moving them to the laboratory where their body mass and head width were measured.

(d) *Reproductive success of female offspring*

The reproductive success of female offspring was defined as the size of their first litter. Most (75%) of the recruits from the first experimental run were already pregnant when trapped in the laboratory at the age of 45 days. These females were allowed to nurse and wean their pups before being released back to the enclosures for winter. All recruits from the second run and the rest (25%) of the first run gave birth to their first litter in spring after overwintering. All pregnant females were moved to the laboratory before parturition. The reproductive success of male offspring was not assessed.

Table 4. *Proportion of individuals (%) that survived from weaning until the next breeding season in relation to offspring number and size manipulations and sex*

(Sample sizes (number of animals returned to enclosures for winter) are in parentheses.)

	number		
size	original	enlarged	total
original	13.7 (51)	23.2 (56)	18.7 (107)
males	4.5 (22)	16.1 (31)	11.3 (53)
females	20.7 (29)	32.0 (25)	25.9 (54)
large	16.1 (31)	28.6 (56)	24.1 (87)
males	23.1 (13)	14.8 (27)	17.5 (40)
females	11.1 (18)	41.4 (29)	29.8 (47)
total	14.6 (82)	25.9 (112)	21.1 (194)
males	11.4 (35)	15.5 (58)	14.0 (93)
females	17.0 (47)	37.0 (54)	27.7 (101)

(e) *Data analysis*

Offspring survival was first examined from a foster mother's point of view by examining the number of offspring alive per litter using three-way ANOVA. The survival probability of individual offspring (survived/died) was investigated using logit models. Variation in offspring body mass and head width was analysed with nested ANOVA where foster mother (random effect) was nested within offspring number and size manipulation and study run (fixed effects). Sex was added to the model if it had a significant effect on body mass or head width. Because of the unbalanced study design, SPSS for Windows (v. 10.0.7) made corrections to nested ANOVA mean squares and *F*-values cannot be calculated directly from tables 1 and 3. Study run was included in all ANOVA models in order to control for possible seasonal differences in conditions between the two runs of the experiment. Tables 1–3 only include the individuals that we were able to measure, whereas table 4 includes all individuals that survived until spring. This caused small differences in the sample sizes.

3. RESULTS

(a) *Reproductive success of mothers*

Litter size was significantly larger in litter size enlargement groups (number) after manipulation and did not differ between the two study runs (run) (two-way ANOVA, run $F_{1,64} = 0.55$ and $p > 0.4$ and number $F_{1,64} = 68.25$ and $p < 0.001$) (figure 1*a*). At weaning, the number of offspring alive per litter was still positively affected by litter size enlargement, but not by offspring size manipulation (size) or their interaction (three-way ANOVA, run $F_{1,62} = 5.81$ and $p = 0.019$, number $F_{1,62} = 10.47$ and $p = 0.002$, size $F_{1,62} = 0.78$ and $p > 0.3$ and interaction $F_{1,62} = 0.03$ and $p > 0.8$) (figure 1*b*). The same effect remained significant at the beginning of the next breeding season (three-way ANOVA, run $F_{1,62} = 1.44$ and $p > 0.2$, number $F_{1,62} = 6.71$ and $p = 0.012$, size $F_{1,62} = 0.002$ and $p > 0.9$ and interaction $F_{1,62} = 0.47$ and $p > 0.4$) (figure 1*c*).

The mean offspring body mass was significantly higher in offspring size manipulation groups after manipulation, but was unaffected by offspring number manipulation or

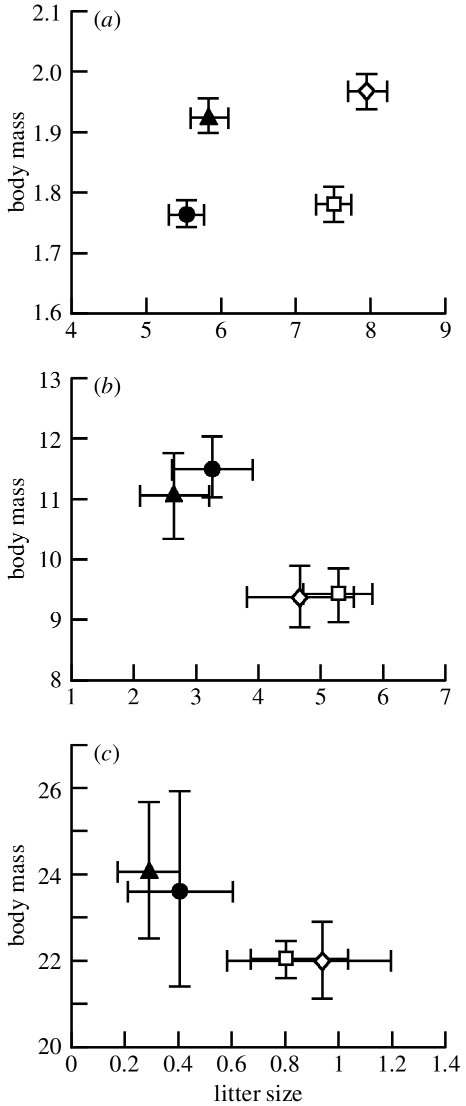

Figure 1. Litter size (number of offspring alive per litter) and offspring body mass (g) in different manipulation groups (*a*) after manipulation, (*b*) at weaning age and (*c*) at the beginning of the next breeding season (mean ± s.e.). Filled circles, offspring number control – offspring size control (number of mothers, $n = 17$); filled triangles, number control–size manipulation ($n = 17$); open squares, number manipulation–size control ($n = 16$); open diamonds, number manipulation–size manipulation ($n = 17$).

the interaction between the two treatments (table 1 and figure 1*a*). By weaning age the effect of size manipulation had disappeared, whereas body mass was significantly lower in enlarged litters (table 1 and figure 1*b*). At the beginning of the next breeding season, offspring number manipulation groups were no longer significantly smaller in body mass, while the situation otherwise remained the same (table 1 and figure 1*c*). The same analyses applied to offspring head width, which is a skeletal measure of body size, gave similar results (tables 2 and 3).

(b) *Survival and reproduction of individual offspring after weaning*

The proportions of individuals surviving from weaning to the next breeding season in relation to offspring number and size manipulations and sex are presented in table 4. The survival probability was analysed using logit models with survival as a dependent variable, and sex and offspring number and size manipulations as explaining factors. The simplest models fitting the data ($p > 0.05$) included sex or offspring number manipulation as the only defining factor. In further analyses offspring sex explained the survival of individuals significantly ($G = 5.48$, d.f. = 1 and $p = 0.019$) with females more likely to survive. There was also a tendency, although non-significant, for the survival of offspring to be enhanced in enlarged litters compared with control litters ($G = 3.36$, d.f. = 1 and $p = 0.067$). The model including only offspring size manipulation did not fit the data ($p < 0.05$). The size of the first litter of female offspring was not related to offspring number or size manipulations (three-way ANOVA, run $F_{1,19} = 0.14$ and $p > 0.7$, number $F_{1,19} = 0.37$ and $p > 0.5$, size $F_{1,19} = 0.51$ and $p > 0.4$ and interaction $F_{1,19} = 2.71$ and $p > 0.1$) (figure 2).

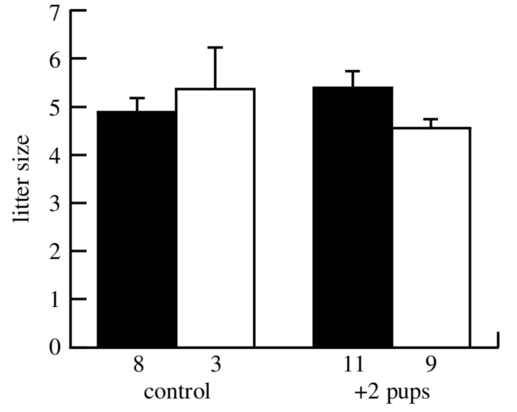

Figure 2. First litter size of female offspring in different manipulation groups (mean ± s.e.). (Black bars, offspring size control group; open bars, offspring size manipulation group. Sample sizes are shown under the bars.)

4. DISCUSSION

The trade-off between offspring size and number is one of the major fitness trade-offs in life-history theory (Stearns 1992). Despite its fundamental importance in the evolution of reproductive effort, there are no experiments on birds or mammals that have addressed the question by manipulating the number and size of offspring simultaneously. We used a novel approach where we artificially enlarged both the offspring number and size of nursing bank vole females and followed the survival and fecundity of offspring until the next breeding season in semi-natural conditions. Our results demonstrated that an enlarged litter size was distinctly advantageous for the mothers since the number of offspring surviving to the next breeding season was higher in enlarged litters than in controls. Offspring body size at weaning was smaller in enlarged litters, which implies that the amount of reproductive effort the mother invests per pup during nursing decreased when litter size was enlarged. However, the manipulation did not seem to have any long-term effect on offspring size as the pups grew to the same size as offspring in other groups after reaching independence of the mother. In contrast, offspring size manipulation did not seem to either improve or impair offspring growth or survival. By weaning age, the effect of offspring size manipulation had levelled off and the mean offspring body mass was also at the same level as the control groups in the following spring. Consequently, it seems that females should be able to increase the number of recruits simply by producing more offspring, whereas increasing offspring size does not benefit either the mother or the offspring.

Based on earlier results, one might expect that litter size enlargement causes a decline in offspring quality (Gustafsson & Sutherland 1988; Dijkstra *et al.* 1990). In the current experiment, the mean offspring body size was smaller in litter size enlargement groups at weaning, but the difference did not persist to the following spring. This suggests that offspring number manipulation affected offspring body size only through the mother's restricted resources and did not decrease the quality of the offspring themselves. When focusing the analyses on individual offspring the results revealed equal survival of offspring in litter size enlargement groups compared with other groups after weaning. Moreover, the reproductive success of female offspring, as measured by the size of their first litter, did not differ between manipulation groups. Therefore, the results suggest that smaller body size at weaning in litter size enlargement groups did not affect the quality of offspring.

The finding that an enlarged litter size does not decrease offspring quality is quite the opposite of some previous studies where this trade-off has been found (Smith *et al.* 1989; Dijkstra *et al.* 1990; Mappes *et al.* 1995; Koskela 1998). However, there is at least one study reporting similar results (Robinson & Rotenberry 1991). Compared with these ones, the current study has one marked refinement. The litter size and offspring size manipulations were combined in the same experiment, whereas previously brood (litter) size manipulations have been performed independently of offspring size. Moreover, the effect of maternal quality on offspring growth was eliminated by using a very careful cross-fostering procedure where all pups in a litter were replaced. Even so, due to yearly and seasonal changes in environmental conditions, our results may not represent the only possible outcome (e.g. Sinervo & DeNardo 1996; Sinervo 1999).

It might be argued that we did not find a trade-off between the number of offspring produced and their quality because current reproductive effort is adjusted according to the demands of future reproduction (Williams 1966). Thereby, females may be able to put more effort into a single reproductive attempt, while in the long run the costs of reproduction ultimately restrict the amount of investment that results in highest lifetime reproductive success. Even though this study focused on only one reproductive attempt there is previous evidence that current reproductive effort is not necessarily maintained by reproductive costs in small mammals (Hare & Murie 1992; Mappes *et al.* 1995; Koskela 1998; Koskela *et al.* 1998, 1999). The costs of reproduction in birds have been found to affect offspring more clearly than parents (Lindén & Møller 1989). However, reproductive costs as well as the trade-off between offspring number and quality may become more evident in unfavourable conditions (e.g. in a poor environment).

In conclusion, it seems that, during evolution, selection directed towards the optimization of offspring size has been substantial in the bank vole since mothers were shown to be unable to rear larger pups more successfully. However, the litter size manipulation demonstrated that females were able to nurse and wean pups from litters larger than they originally gave birth to and that individual offspring survived equally well in these enlarged litters. Thus, our results could be interpreted as evidence for higher selective pressure against larger offspring size than against larger litter size, possibly due to more severe restrictions, e.g. of physiological nature. Thus, offspring size may be evolutionarily more fixed than litter size, and phenotypic variation in the size of the offspring does not appear to affect reproductive success.

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