HORMONAL MANIPULATION OF OFFSPRING NUMBER: MATERNAL EFFORT AND REPRODUCTIVE COSTS

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Abstract.—We used exogenous gonadotropin hormones to physiologically enlarge litter size in the bank vole (*Clethrionomys glareolus*). This method allowed the study design to include possible production costs of reproduction and a trade-off between offspring number and body size at birth. Furthermore, progeny rearing and survival and postpartum survival of the females took place in outdoor enclosures to capture salient naturalistic effects that might be present during the fall and early winter. The aim of the study was to assess the effects of the manipulation on the growth and survival of the offspring and on the reproductive effort, survival, and future fecundity of the mothers. Mean offspring body size was smaller in enlarged litters compared to control litters at weaning, but the differences disappeared by the winter. Differences in litter sizes disappeared before weaning age due to higher mortality in enlarged litters. In addition to the effects of the litter size, offspring performance was probably also influenced by the ability of the mother to support the litter. Experimental females had higher reproductive effort at birth, and they also tended to have higher mortality during nursing. Combined effects of high reproductive effort at birth and high investment in nursing the litter entailed costs for the experimental females in terms of decreased probability of producing a second litter and a decreased body mass gain. Thus, enlarged litter size had both survival and fecundity costs for the mothers. Our results suggest that the evolution of litter size and reproductive effort is determined by reproductive costs for the mothers as well as by a trade-off between offspring number and quality.

Key words.—*Clethrionomys glareolus*, cost of reproduction, gonadotropin, litter size manipulation, offspring size, reproductive effort, trade-off.

A number of brood size manipulation studies have shown that birds can rear more young than their average clutch size (see for review Lindén and Møller 1989; VanderWerf 1992; Monaghan and Nager 1997). These results have made the evolution of optimal offspring number one of the most puzzling topics in the field of life-history research. Selective constraints such as the costs of reproduction (i.e., reduced survival or fecundity) have been presumed to offer an explanation for this discrepancy (e.g., Williams 1966; Lindén and Møller 1989). However, it is difficult to find any general empirical pattern for the determinants of optimal offspring number, because important factors, such as the trade-off between offspring number and size and production costs of reproduction, have not been integrated into experimental offspring number manipulation procedures. The physiological causes of life-history trade-offs have been studied for decades (Roff 1992; Stearns 1992), but the importance of the mechanisms underlying them has not been recognized until recently (Ketterson and Nolan 1992, 1999; Finch and Rose 1995; Sinervo 1999; Zera and Harshman 2001).

The trade-off between offspring number and size at birth has been detected in several vertebrates including birds, mammals, and lizards (e.g., Kaufman and Kaufman 1987; Robinson and Rotenberry 1991; Sinervo and Licht 1991a). Offspring number manipulations will often create unnatural situations due to this negative covariance, because females cannot necessarily increase the number of offspring without simultaneously decreasing the birth size of individual offspring. Thus, when a trade-off between offspring number and size exists, the manipulation of offspring number should be combined with a simultaneous manipulation of mean offspring size (Sinervo and Licht 1991a). Many fitness-related

offspring performance traits (i.e., reproductive success or survival) have often been found to be size dependent; thus, offspring size at birth may be an important component contributing to optimal reproductive effort (Roff 1992; Sinervo and DeNardo 1996).

Rearing a large number of offspring requires more parental effort not only when feeding the young, but also when producing them (Monaghan and Nager 1997). In some bird species there are measurable costs associated with laying and incubating eggs (Heaney and Monaghan 1996; Monaghan et al. 1998; Cichoń 2000). This may be a common confounding factor in the plethora of offspring number manipulation studies. Even if parents can rear an artificially enlarged number of offspring successfully, they might not have been able to both produce and rear the additional offspring. In many species of birds, it is possible to manipulate females to lay additional eggs. Moreover, incubation costs can be taken into account in offspring number manipulations by adding or removing eggs instead of newly hatched chicks (Heaney and Monaghan 1995; Visser and Lessells 2001). In mammals, however, including the production costs into a study design is far more complicated.

To include the above aspects into an offspring number manipulation experiment requires that females be manipulated to produce more offspring than usual and to simultaneously reduce the size of individual offspring. Even though most of the traditional brood size manipulations have been performed in birds, methods combining offspring number and size manipulations have been recently developed for lizards (Sinervo 1999). Sinervo and Licht (1991b) have successfully used follicle stimulating hormone (FSH) to increase mean clutch size with a simultaneous decrease in egg mass in side-

blotched lizards (*Uta stansburiana*). Similar methods have been widely used in laboratory mammals to produce a large number of eggs (superovulation) for embryonic research (e.g., Edirisinghe et al. 1986; Ziebe et al. 1993; Muñoz et al. 1995). In evolutionary ecology, superovulation techniques have been applied in embryo transfer experiments to study the effects of prenatal maternal effects on phenotypic evolution (e.g., Cowley et al. 1989; Cowley 1991). However, these experimental methods have not been previously used to study the evolutionary implications of life-history phenomena in mammals.

Previous litter size manipulation studies carried out in small mammals suggest that the fitness of the mother, in terms of offspring quality, is not increased by postnatal manipulation in either direction (Kaufman and Kaufman 1987; Koskela 1998). Moreover, no effects of the manipulation have been found on the survival or future fecundity of the mothers (Hare and Murie 1992; Mappes et al. 1995; Humphries and Boutin 2000; Neuhaus 2000). Therefore, the conclusion derived from previous litter enlargement and reduction experiments in small mammals is that females do not trade off their own condition against the quality of their offspring. In this paper, we present the effects of physiologically enlarging litter size on the performance of the offspring and the mothers. The litter size of bank vole females was manipulated by injecting them with exogenous gonadotropin hormones to induce development of a greater number of embryos than normal. This method enabled us to manipulate litter size and offspring size simultaneously and to include the production costs into our study design. 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. The performance of the offspring was measured as the number and quality (body size and condition) of offspring reared to independence and in the following winter. Reproductive effort, body mass, survival, and the probability to produce a second litter were used as measures of reproductive costs for the mothers.

In mammals, offspring survival during nursing is dependent on mother survival, and thus selection should favor females who can keep the survival costs of reproduction constant over small changes in their reproductive effort (Tuomi 1990). This may have hindered the detection of reproductive costs in studies where offspring number has been traditionally manipulated (e.g., Hare and Murie 1992; Mappes et al. 1995; Humphries and Boutin 2000). The hormonal manipulation of reproductive effort generates additional phenotypic variation for selection to act on, and thus enhances the detection of reproductive costs (Sinervo and DeNardo 1996; Miles et al. 2000; Zera and Harshman 2001). This enables the significance of reproductive costs on the evolution of optimal reproductive effort to be studied.

MATERIALS AND METHODS

Study Site and Study Species

The study was conducted at Konnevesi, central Finland (62°37′N, 26°20′E). The experiment was carried out in two separate identical trials: the first in June–July (eight enclosures) and the second in August–September 1999 (11 enclo-

sures). Subsequent offspring performance was followed from September to December 1999 (10 enclosures). The manipulation of reproductive effort was performed in the laboratory, where the animals were housed in standard mouse cages measuring $43 \times 26 \times 15$ cm and maintained in a 16L:8D photoperiod. Wood shavings were provided as bedding and food and water were available continuously.

After manipulation, the experiment continued in 11 outdoor enclosures (0.2 ha) situated in a field. 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 and sunflower seeds. Enclosure fences were constructed of 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 prevent possible entry of predators (e.g., red fox, *Vulpes vulpes*; least weasel, *Mustela nivalis nivalis*; or avian predators), particularly in winter when the snow cover was high.

The study species, the bank vole (*Clethrionomys glareolus*), is a common mammalian species in northern Europe. Females give birth to between two and 10 pups per litter and can have up to four litters in a breeding season (M. Koivula, E. Koskela, T. Mappes, and T. A. Oksanen, unpubl. data). The nursing period lasts around 20 days, after which the pups reach total independence from their mother. The individuals used in the experiment 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 the experiment started.

Manipulation

We applied the method used by Muñoz et al. (1995) to increase the ovulation rate in mature bank vole females. Gonadotropin from human menopausal urine (hMG; Sigma Chemical Co., St. Louis, MO) was used to generate an ovulation of more follicles than normal and human chorionic gonadotropin (hCG; Sigma) was used to induce ovulation. The effect of the hMG is ephemeral, and therefore, it is important that the females ovulate before the hormone disappears from the their systems. Experimental females were treated with hMG, which contains approximately equal portions of follicle stimulating hormone (FSH) and luteinizing hormone (LH). Females were subcutaneously injected five times once every 12 h with 0.5 IU hMG dissolved in 0.1 ml isotonic sodium chloride. The dose was calculated on the basis of the FSH content, because FSH is the principal regulator of follicular growth and maturation, whereas the importance of exogenous LH in ovarian stimulation is questionable (Lunenfeld and Lunenfeld 1997; Howles 2000). Twelve hours after the last injection the females were injected with 5 IU of hCG dissolved in 0.1 ml isotonic sodium chlo-

ride. Control females received injections of parallel amounts of isotonic sodium chloride during the same intervals as the experimental group. After the last injection, both control and experimental females were paired to randomly chosen mature males and each pair was placed in the same cage for a week. All females in the experiment were weighed with an electronic scale before the pairing. There was no difference in mean female body mass between the treatments (mean ± SE, control: 20.6 ± 0.7 g; gonadotropin: 21.1 ± 0.9 g; $t = -0.42$, $n = 38$, $P > 0.6$).

Offspring Quality and Reproductive Effort of Mothers

Females were observed twice a day to record parturition. When the birth was detected, pups were sexed, individually marked, weighed with electronic scales, and their head width was measured under a microscope. Standardized residuals of the regression of body mass on head width were used as condition index describing the nutritional state of offspring (e.g., Dobson and Michener 1995; Jakob et al. 1996; Kotiaho 1999). The mothers were weighed and then paired again in postpartum estrus with a randomly chosen male to estimate their probability of producing a second litter. The pairs were observed in a behavioral arena to ensure that all females mated and thus had an equal opportunity to produce the second litter. After measurements in the laboratory, females and their offspring were transferred to the outdoor enclosures in their breeding cages. All individuals released into the enclosures were individually marked for later identification. One control mother (first trial $n = 8$, second trial $n = 11$) and one experimental mother (first trial $n = 8$, second trial $n = 11$) were placed near the opposite corners of each enclosure (in rainproof covers) and their breeding cages were left open so the 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; Oksanen et al. 2001).

After weaning their pups (about 25 days from parturition), the mothers were trapped twice a day until no new individuals were caught and taken to the laboratory to weigh their body mass and to check whether they were pregnant with a second litter. Reproductive effort (RE) of mothers was estimated at birth and at weaning. We used relative litter mass as an estimate of RE:

$$\text{RE} = LM_o/M_m, \qquad (1)$$

where L is the number of offspring alive (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 represents the effort during the pregnancy, whereas the estimate of RE at weaning represents the effort invested in nursing the offspring.

At the age of 30 days, all the pups were trapped following the same method as for the mothers and taken to the laboratory to weigh body mass, measure head width, and to determine their survival to weaning age. The head width of the juveniles was measured with a digital caliper. The second trial, which was identical to the first one but used different females, was subsequently started in empty enclosures. In September, after the second trial was completed, all offspring (from both trials) were randomly distributed among 10 en-
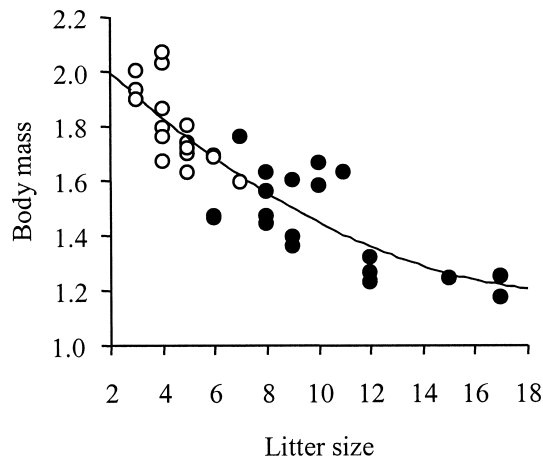

Fig. 1. Litter size (number of offspring per litter) and mean offspring body mass (g) at birth in control litters (open circles) and in enlarged litters (closed circles; $y = 0.002x^2 - 0.098x + 2.18$, $n = 38$, $P < 0.001$).

closures to study their performance further (eight or nine offspring per enclosure). In December, the growth and survival of these individuals was determined following the same methods that were used at age 30 days.

Data Analysis

We used two-way ANOVAs, logistic regressions, and Pearson chi-square tests to analyze the data. The unit for sample size is the number of mothers or the number of individual offspring (Pearson chi-square test and logistic regression), and the mean value of offspring in the same litter (ANOVA models). Study trial was included in all ANOVA models, because of its significant effect in most of the analyses, but excluded from the logistic regressions, where it had no effect at all. Offspring survival from weaning to winter was analyzed for individuals alive in September when they were released back to enclosures. Analyses of deviance in logistic regressions were conducted according to Hardy and Field (1998), and effect size and the power of the test for mother survival were analyzed according to Faul and Erdfelder (1992). Only the females that survived through the first nursing period were included in the analysis of probability of producing a second litter.

RESULTS

Offspring Growth and Survival

Females receiving hMG and hCG treatment produced significantly larger litters (two-way ANOVA, trial: $F_{1,32} = 0.23$, $P > 0.6$; treatment: $F_{1,32} = 85.58$, $P < 0.001$) consisting of smaller offspring (two-way ANOVA, trial: $F_{1,32} = 0.53$, $P > 0.4$; treatment: $F_{1,32} = 28.35$, $P < 0.001$) compared to control females (Fig. 1). There was no difference in offspring sex ratio between the control litters and the enlarged litters (mean proportion of males ± SE, control: 0.46 ± 0.05, enlarged: 0.54 ± 0.05, two-way ANOVA for arcsine-transformed values, trial: $F_{1,35} = 1.45$, $P > 0.2$; treatment: $F_{1,35} = 1.05$, $P > 0.3$). Three gonadotropin-treated females that produced more than 12 pups were excluded from the statis-
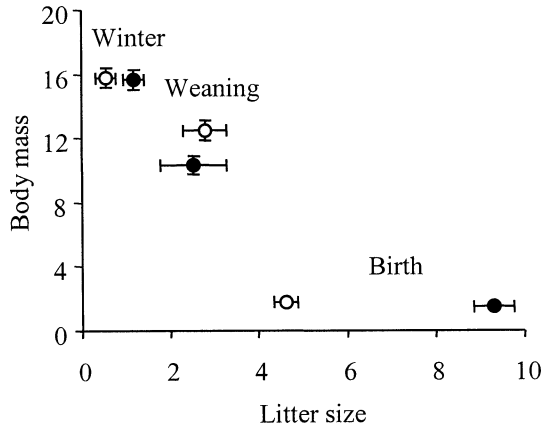
FIG. 2. Litter size (number of offspring alive per litter) and mean offspring body mass (g) in control litters (open circles) and in enlarged litters (closed circles) at birth, at weaning, and in winter (mean ± SE).

tical analyses (Fig. 1) because we did not want to exceed the natural maximum of 10 by more than two pups. In our previous litter size manipulation studies, in which pups are added or removed from a litter, we have restricted manipulation to a limit of two pups around the natural maximum (Koskela 1998; Koskela et al. 1998, 1999; Oksanen et al. 2001).

Despite larger litter size at birth, the number of offspring alive per litter at weaning was not larger in experimental females than in control females (two-way ANOVA, trial: $F_{1,32} = 0.09$, $P > 0.7$; treatment: $F_{1,32} = 0.12$, $P > 0.7$; Fig. 2). However, mean offspring body mass remained smaller in enlarged litters at weaning (two-way ANOVA, trial: $F_{1,20} = 1.61$, $P > 0.2$; treatment: $F_{1,20} = 5.95$, $P = 0.024$; Fig. 2). In winter, the treatments did not differ in the number of offspring alive per litter (two-way ANOVA, trial: $F_{1,32} = 0.97$, $P > 0.7$; treatment: $F_{1,32} = 0.12$, $P > 0.7$; Fig. 2) or in mean offspring body mass (two-way ANOVA, trial: $F_{1,15} = 8.30$, $P = 0.011$; treatment: $F_{1,15} = 0.74$, $P > 0.4$; Fig. 2). Analyses on mean offspring head width, a skeletal measure of body size, gave results consistent with those for body mass (Tables 1, 2). Offspring condition was also significantly lower in enlarged litters at birth, but the differences disappeared before weaning and winter (Tables 1, 2).

We tested the effects of treatment and body mass at birth on offspring survival using analysis of deviance in logistic regression. Offspring survival during nursing was significantly lower in enlarged litters than in control litters (percentage of survived individuals, control: 60.2%, $n = 88$; enlarged: 30.8%, $n = 130$; Table 3). Moreover, high body mass

at birth tended to have a positive effect on the survival through the nursing period (mean body mass at birth ± SE, dead: 1.55 ± 0.02, $n = 124$; survived: 1.67 ± 0.02, $n = 93$; Table 3). Offspring survival from weaning to winter was lower in individuals from enlarged litters as well (percentage of survived individuals, control: 43.4%, $n = 53$; enlarged: 22.5%, $n = 40$; Table 3). Body mass at birth, however, was not related to the survival of the individuals from weaning to winter (mean body mass at birth ± SE, dead: 1.65 ± 0.03, $n = 61$; survived: 1.70 ± 0.03, $n = 32$; Table 3).

Maternal Effort and Reproductive Costs

Reproductive effort (RE) at birth was significantly higher in experimental females compared to control females (mean ± SE, control: 0.37 ± 0.02, $n = 19$; enlarged: 0.60 ± 0.02, $n = 16$; two-way ANOVA, trial: $F_{1,32} = 0.03$, $P > 0.8$; treatment: $F_{1,32} = 68.83$, $P < 0.001$). When compared with the number of offspring alive per litter in winter, the mean RE of the control mothers was closer to the most productive value of RE at birth (0.399) than the mean of the experimental mothers (Fig. 3). RE during weaning did not differ between the treatments (mean ± SE, control: 1.52 ± 0.26, $n = 19$; enlarged: 1.06 ± 0.30, $n = 16$; two-way ANOVA, trial: $F_{1,32} = 0.09$, $P > 0.7$; treatment: $F_{1,32} = 1.28$, $P > 0.2$). However, experimental mothers tended to have lower survival through the nursing period compared to control females (percentage of survived individuals, control: 89.5%, $n = 19$; enlarged: 62.5%, $n = 16$; $G = 3.67$, df = 1, $P = 0.055$; effect size = 0.32, power of the test = 0.47). Even so, the probability of a complete litter loss did not differ significantly between the groups (percentage of lost litters, control: 26.3%, $n = 19$; enlarged: 43.8%, $n = 16$; Pearson chi-square: $\chi^2 = 1.172$, df = 1, $P = 0.279$).

The body mass of the mother increased in both treatments during the experiment (mean increase ± SE [g], control: 1.5 ± 0.3, $n = 17$; enlarged: 2.4 ± 0.9, $n = 10$). Body mass increase was negatively related to RE at weaning in the experimental group (linear regression, $y = -1.71x + 5.26$, $n = 10$, $P = 0.019$), but not in control females (linear regression, $y = 0.07x + 1.37$, $n = 17$, $P > 0.8$). Comparison of regression coefficients revealed that the slopes of the equations were significantly different ($t = 2.97$, df = 23, $P = 0.007$). We used a logistic regression to test whether RE at weaning and/or treatment affected the probability that females produced a second litter. The analysis of deviance revealed that there was a significant interaction between RE and treatment, and thus the proportion of second breeders tended to decrease with high RE at weaning in the experi-

TABLE 1. Descriptive statistics for offspring head width (mm) and condition (standardized residuals from the regression of body mass on head width) from parturition to winter in relation to litter size manipulation.

| | Head width | | | | | | Condition | | | | | |
| | Control | | | Enlarged | | | Control | | | Enlarged | | |
	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
Birth	8.10	0.07	19	7.74	0.08	16	0.43	0.23	19	−0.36	0.20	16
Weaning	12.60	0.13	14	11.70	0.11	9	−0.28	0.23	14	0.43	0.27	9
Winter	13.20	0.11	12	13.16	0.14	6	−0.10	0.31	12	0.21	0.31	6

TABLE 2. Offspring head width and condition (standardized residuals from the regression of body mass on head width) from parturition to winter in relation to litter size manipulation (two-way ANOVA with treatment and study trial as explaining factors).

	At birth				At weaning				In winter			
	df	MS	F	P	df	MS	F	P	df	MS	F	P
Head width												
Trial	1	1.13	19.21	<0.001	1	0.24	1.31	0.265	1	0.24	1.80	0.199
Treatment	1	1.21	20.63	<0.001	1	4.00	21.60	<0.001	1	0.01	0.01	0.908
Error	32	0.05			20	0.18			15	0.13		
Condition												
Trial	1	12.83	28.56	<0.001	1	0.77	0.69	0.356	1	5.91	9.15	0.009
Treatment	1	4.42	9.83	0.004	1	3.18	3.65	0.070	1	1.38	2.15	0.163
Error	32	0.44			20	0.87			15	0.64		

mental group and to increase in the control group (Table 4, Fig. 4).

DISCUSSION

In this study, we used exogenous gonadotropin hormones to generate additional phenotypic variation in the litter size in bank vole females. The method enabled us to overcome some restrictions of the traditional brood size manipulations and to obtain a new perspective on the study of optimal reproductive effort and the costs of reproduction (Williams 1966). At birth, the litters of the gonadotropin-treated females were significantly larger and consisted of smaller individual offspring than the litters of the control females, but there was no difference in the sex ratios of the litters. Mean offspring body size at weaning was smaller in enlarged litters, but the differences disappeared before the winter. Offspring condition at birth was significantly lower in enlarged litters, but no effect of manipulation was found at weaning or in winter. Thus, the nutritional state of the offspring seemed to improve during nursing, although mean offspring size remained smaller. Mortality during the nursing period was higher in enlarged litters, and there was no difference in the number of offspring alive per litter at weaning. However, whole litter loss was not more common in the mothers nursing enlarged litters, suggesting that they were not doomed to failure. The mortality remained significantly higher in enlarged litters from weaning to winter, but did not cause any further differences

TABLE 3. Analysis of deviance in logistic regression for the probability of the offspring to survive from birth to weaning ($n = 217$) and from weaning to winter ($n = 93$). Treatment and body mass at birth are in model as covariates. Full–*variable* indicates full model without the variable in question. Full model includes the intercept, treatment, and body mass.

Model	Deviance	G	df	Tested for	P
Birth-weaning					
Intercept only	296.38	21.35	2	full model	<0.001
Full – body mass	278.76	3.74	1	body mass	0.052
Full – treatment	282.31	7.30	1	treatment	0.006
Full model	275.02				
Weaning-winter					
Intercept only	119.73	4.62	2	full model	0.099
Full – body mass	115.12	0.01	1	body mass	0.920
Full – treatment	118.80	3.69	1	treatment	0.054
Full model	115.11				

in litter sizes between the treatments. Our results are consistent with Williams (2001), who reported that zebra finch females (*Taeniopygia guttata*) treated with anti-estrogen tamoxifen produced more eggs, but had lower relative hatching success than control females. Consequently, there was no difference between the physiologically manipulated females and the control females in the number of chicks fledged or in the body size of these chicks. In the current study, low body mass at birth had a negative effect on offspring survival from birth to weaning, but not during the following period from weaning to winter. Therefore, the effect of body mass at birth on offspring survival may have been partly mediated through other circumstances during the nursing period, such as the quality of maternal care. Assuming that the effort invested in each individual offspring decreases with increasing litter size, it is possible that the disadvantages of small body size at birth were not overcome during nursing due to restrictions in the mother's ability to nurse her litter.

Although it seems intuitively reasonable that the factor restricting a mother's ability to nurse the litter is the production of milk, this is not necessarily the case. It is well known that in mammals with variable litter size, prepartum mammogenesis is hormonally adjusted to the number of growing embryos (e.g., Jameson 1998). Moreover, hormon-
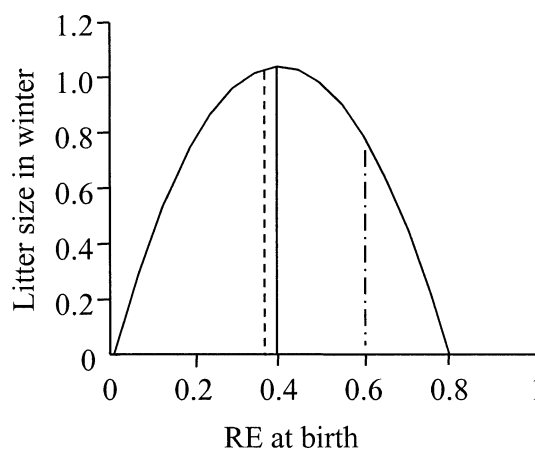

FIG. 3. Reproductive effort (RE) at birth and the number of offspring alive per litter in winter (quadratic regression of RE on litter size in winter, $y = -6.556x^2 + 5.230x$, $n = 35$, $P < 0.001$). Solid vertical line, the most productive value of RE at birth; dashed line, mean value of control mothers; dotted dashed line, mean value of experimental mothers.

TABLE 4. Analysis of deviance in logistic regression for the probability of the mother to produce a second litter ($n = 27$). Treatment and reproductive effort (RE) at weaning are in the model as covariates. Full–*variable* indicates full model without the variable in question. Full model includes the intercept, treatment, RE, and interaction.

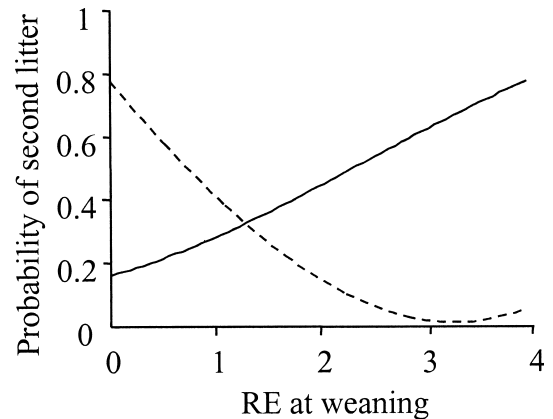| Model	Deviance	G	df	Tested for	P
Intercept only	35.59	6.06	3	full model	0.109
Full – RE	33.34	3.75	1	RE	0.053
Full – treatment	32.44	2.85	1	treatment	0.091
Full – treatment × RE	35.25	5.66	1	treatment × RE	0.017
Full model	29.60				

FIG. 4. Reproductive effort (RE) at weaning and the predicted probability of the mothers to produce a second litter (from logistic regression of treatment, RE at weaning and interaction on production of a second litter). Solid line, control females line ($y = 0.005x^3 + 0.034x^2 + 0.092x + 0.162$, $n = 17$, $P < 0.001$); dashed line, experimental females ($y = 0.006x^3 + 0.032x^2 - 0.402x + 0.775$, $n = 10$, $P < 0.001$).

ally induced superovulation may increase the activity of the mammary glands during lactation (Frimawaty and Manalu 1999). Therefore, one of the advantages of the hormonal manipulation method over the traditional litter size manipulation is that it ensures better mammogenesis. Furthermore, the reproductive costs of such endocrine-mediated events are only possible to assess with endocrine manipulation of reproductive effort. Thus, it is not self-evident that the smaller body size at weaning and higher mortality of the offspring in experimental litters was caused by a decreased milk yield per pup. The restricting factor may be competition among the siblings or some other component of maternal care. In small mammals food intake may be more than doubled during lactation, and in many cases the magnitude of the increase in food intake is positively related to the number of young being suckled (see review by Wade and Schneider 1992). A positive relationship between litter size and daily food consumption has also been found in the bank vole (Oksanen et al. 1999). It is possible, therefore, that the higher mortality of experimental pups was due to their mothers spending more time away from the nest foraging, and thus leaving the offspring without maternal care (e.g., nest defense, lactation, thermoregulation) for longer periods of time than the control mothers.

Litter production required more effort from experimental females because their RE at birth was higher than that of control females. The RE at birth of the control mothers resulted, on average, in a larger number of offspring alive per litter in the following winter than the RE of the experimental mothers. Moreover, the mean RE of the control mothers was only slightly lower than the most productive value of RE at birth. In contrast, the mean RE of the experimental mothers was far higher than the most productive value of RE at birth. Therefore, the ratio between the effort invested in and the expected outcome of the reproductive attempt was poor in experimental mothers. RE at weaning did not differ between the treatments because the differences in litter sizes had already leveled off.

Mortality was higher in experimental mothers during the nursing period, although the statistical power of our test was not necessarily strong enough to detect a significant effect (Cohen 1988). Similarly, Sinervo and DeNardo (1996) and Miles et al. (2000) report that in side-blotched lizards (*U. stansburiana*) current reproductive investment has costs in terms of lowered survivorship to the next clutch. Our results showing an increase in mean body mass of the mothers during

nursing in both treatments support the common phenomenon that small mammals increase their food consumption rather than deplete body reserves during lactation (Sikes 1995; Humphries and Boutin 1996). An increase in body mass was negatively related to RE at weaning in experimental females, 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. A similar trend was observed in the future fecundity of the mothers. High RE at weaning decreased the proportion of second breeders in the experimental group and increased their proportion in the control group. Thus, the fecundity costs of high RE at weaning could be seen in the experimental females, who also had higher RE at birth, whereas the control females seemed to be able to bear the costs of high RE at weaning and were more likely to produce a second litter. Therefore, the cost of reproduction, in terms of both future fecundity and gain in body mass, seemed to be a joint effect of high RE at birth and at weaning. Identifying such an effect would not have been possible by simple litter enlargement and reduction manipulation.

Compared to the knowledge gained from earlier litter size manipulation studies in small mammals, the findings of the current study are consistent with those suggesting that offspring survival and body size at weaning decreases with increasing offspring number (Kaufman and Kaufman 1987; Koskela 1998). However, litter enlargement and reduction experiments have failed to find survival or fecundity costs of reproduction, suggesting that mothers do not trade-off their own condition for the quality of their offspring (Hare and Murie 1992; Mappes et al. 1995; Humphries and Boutin 2000; Neuhaus 2000). In this respect, the results from the current hormonal manipulation experiment—implying that the costs of reproduction may be an even more important factor in determining the optimal reproductive effort than the

trade-off between offspring number and size—are significant. The differences in the conclusions from studies with different manipulation methods indicate that adopting new perspectives may be helpful in approaching the dilemmas of even the most extensively studied evolutionary theories, such as life-history theory.

In conclusion, mothers producing large litters of small individual offspring did not gain any fitness benefits in terms of the number or size of offspring alive per litter in the following winter. On the contrary, the results suggest that enlarged litter size had both survival and fecundity costs for the mothers. Moreover, in addition to the effects of the litter size, offspring performance in large litters may have been determined by the ability of the mother to support the litter. Our results show that even under conditions that favor high reproductive effort, the evolution of litter size will be restricted by reproductive costs for the mothers. Previously, survival and fecundity costs of reproduction have turned out to be difficult to detect in mammals (Hare and Murie 1992; Mappes et al. 1995; Koskela 1998; Neuhaus 2000). This may be because simple enlargement and reduction experiments do not capture the full scope of endocrine-triggered costs associated with the ovarian regulation of large litters. In this study, we expanded the range of normal variation in litter size with manipulations of ovarian regulation and were able to reveal the significance of the reproductive costs on the evolution of optimal reproductive effort.

Among rodents, a large number of species produce a highly variable litter size. The most common patterns of such variation result from age and reproductive history of the mother and from environmental fluctuations (Jameson 1998). In voles, life-history traits show strong plasticity, which may be highly advantageous in fluctuating environments (Ergon et al. 2001). The maternal effect hypothesis suggests that mothers may respond to changes in environment by modifying the characteristics of their offspring (Rossiter 1996; Mousseau and Fox 1998). Therefore, maternal effects may provide mechanisms for adapting to variable environments and even explain population cycles in small mammals (Boonstra and Hochachka 1997; Inchausti and Ginzburg 1998). Potential for maternal effects is large in mammals, because the females nurse their offspring, and the evolutionary implications of such effects on life-history evolution may be substantial. In the future, rigorous experimental designs are needed to understand the role of maternal effects in the evolution of life-history traits in mammals.

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