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Manipulation of offspring number and size: benefits of large body size at birth depend upon the rearing environment

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Summary

1. Allocation of reproductive effort between the number and size of offspring determines the immediate rearing environment for the growing young. As the number of offspring increases, the amount of parental investment per individual offspring decreases, and the quality of the rearing environment is expected to decrease. This may result in a lower quality of offspring reared in such conditions.

2. We studied the effects of the rearing environment on the quality of juvenile bank voles, *Clethrionomys glareolus*, with different initial body sizes at birth in a 2×2 factorial experiment. The rearing environment was manipulated by enlarging both the litter size by two extra pups, and mean offspring body size at birth by replacing the original litter with heavier pups from smaller litters. Offspring quality was estimated from body size measurements, parasitic infection with *Eimeria* spp. and the level of immune response to a novel antigen.

3 The analyses revealed that large body size at birth was an advantage in 'normal' rearing environments, but a disadvantage in poor ones. The initially normal sized offspring grown in enlarged litters had a relatively good capacity for growth and high immune function confirming that a poor rearing environment alone does not reduce their quality.

4 Our findings that the benefits of large body size depend on the rearing environment suggest that offspring body size is adjusted in relation to litter size, and thus the evolution of these two traits is combined.

Key-words: *Clethrionomys glareolus*, immune response, life history trade-offs, litter size manipulation, maternal effects.

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Introduction

The environment experienced during ontogeny can have important consequences for the subsequent performance of individuals, and the evolution of life-history traits (Lindström 1999). There is widespread experimental evidence for the influence of environmental variables such as temperature, altitude or diet on offspring body mass, morphology, growth rate, sexual ornaments and mortality (e.g. Sorci, Clobert & Belichon 1996; Shine *et al*. 1997; Birkhead, Fletcher & Pellat 1999; Ohlsson *et al*. 2002). General environmental factors like these often influence the whole population and thus their potential for generating genetic variation among individuals is relatively weak. Differences in parental phenotypes, however, may function as more specific environmental effects that generate individual variation in early development, and influence the evolution of life-history traits (Rossiter 1996; Mousseau & Fox 1998). For example, the nutritional, hormonal and metabolic environment provided by the mother modifies the growth and development of offspring during the foetal period (Cowley *et al*. 1989; Desai & Hales 1997). Moreover, many organisms, such as most birds and mammals, are dependent on parental care during their early life stages, and the quality of the parents, together with the characteristics of the brood/litter, form the most immediate environment for the growing offspring.

As parents have to allocate their reproductive effort among their offspring, the amount of investment per individual decreases with increasing number of offspring,

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and thus, the quality of the rearing environment is also expected to decrease (Lack 1947; Smith & Fretwell 1974). Variation in the rearing environment may affect the subsequent performance of the offspring. This prediction is equivalent to the trade-off between offspring number and quality (e.g. body size or survival) as predicted by life-history theory (Roff 1992). Moreover, it is consistent with the negative maternal effect hypothesis, which suggests that large females produce large litters of small, slow-growing offspring and vice versa (Leamy 1981; Millar 1982). Recently, studies suggesting that the immune system may function in mediating the costs of various energetically demanding activities, have added a new dimension to this reasoning (Sheldon & Verhulst 1996). If individuals growing in poor environments have less available energy, then balancing an energy budget between the most essential bodily functions becomes challenging. Conclusions from the previous offspring number manipulation studies support this reasoning. The costs experienced in poor environments are decreased body size and lower immune capability or survival (e.g. Gustafsson & Sutherland 1988; Dijkstra *et al*. 1990; Koskela 1998; Hõrak *et al*. 1999; Humphries & Boutin 2000). However, survival costs have not always been detected (Oksanen *et al*. 2001), and, at least in one bird species, the physiological and biochemical development seems to be relatively invariant with respect to brood size manipulation (Burness *et al*. 2000).

The number of the offspring, however, is not the only factor parents have to consider with regards to their reproductive investment. The body size of individual offspring may be as important, and therefore the dilemma of their optimal reproductive effort actually concerns the allocation of effort between the number and size of offspring (Smith & Fretwell 1974). In general, offspring size in relation to offspring quality has been studied far more infrequently than offspring number. There are some experimental studies which report that in birds heavier eggs tend to produce heavier offspring, but later in the breeding season offspring performance seems to be more dependent on environmental factors and parental quality than on the size of the egg (e.g. Reid & Boersma 1990; Bolton 1991; Blomqvist, Johansson & Götmark 1997). Moreover, it has been shown that the quality of the egg is, in general, a better measure of parental investment than is egg size (Nager, Monaghan & Houston 2000). In mammals, the significance of early body size in terms of future performance has been studied in relatively few species (e.g. Koskela 1998; Kruuk *et al*. 1999; Festa-Bianchet, Jorgenson & Réale 2000; Neuhaus 2000), but to date, the experimental approach of manipulating body size is almost completely lacking in studies on life history trade-offs (Oksanen *et al*. 2001; Oksanen, Koskela & Mappes 2002).

Previously, we conducted a 2×2 factorial experiment where maternal effort of female bank voles (*Clethrionomys glareolus* Schreber) was manipulated by replacing the original litter with heavier pups from smaller litters, and/or enlarging the litter size by two extra pups (Oksanen *et al*. 2001). Results from this experiment suggested that mothers nursing enlarged litters had, on average, more offspring alive at the beginning of the next breeding season compared to mothers nursing control litter sizes, although the mean body size of offspring at weaning was smaller. Manipulation of the offspring body size, on the other hand, did not have any effect on the growth or survival of the offspring. In this paper, we aim to investigate the mechanisms behind these results by analysing the effects of the rearing environment (litter size manipulation) on the quality of individual offspring with different initial body sizes at birth. The measures of offspring quality included: serum IgG, white blood cell count, haematocrit, humoral immune response to a novel antigen measured from blood samples, intensity of parasite infection (*Eimeria* spp.) and the gain in body size three weeks after becoming independent from their mother. In particular, we were interested in whether the effects of offspring body size on their future performance were influenced by the quality of their rearing environment, and if such maternal environmental effects have the potential to modify the evolution of life-history traits.

Materials and methods

STUDY SITE AND STUDY SPECIES

The outdoor study was conducted at Konnevesi, central Finland (62°37′ N, 26°20′ E), in 11 0·2 ha enclosures situated in an old field. Two separate runs of the experiment were carried out in 1998: the first in June–July (11 enclosures) and the second in July–August (9 enclosures). 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 that 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 (e.g. red fox *Vulpes vulpes* L., least weasel *Mustela nivalis nivalis* L. and avian predators). During the outdoor study, the animals were dependent on naturally occurring food resources. In the laboratory the animals were housed in standard mouse cages measuring $43 \times 26 \times 15$ cm and maintained in a 16 : 8 h light/dark photoperiod. Wood shavings were used as bedding and food pellets and water were continuously available.

The study species, the bank vole, gives birth to a maximum of four litters during the breeding season, with the litter size ranging from 1 to 10. The breeding season lasts from late April to September and pups become independent from their mother before the age of three weeks. Females are unable to distinguish their

own pups from foreign ones, which enables litter manipulations and cross-fostering of new-born offspring (Mappes, Koskela & Ylönen 1995). Individuals used in the experiment were second generation laboratory born descendants of wild bank voles originally captured close to the study site. All individuals had reproduced at least once before the experiment.

MANIPULATION OF REARING ENVIRONMENT AND BODY SIZE AT BIRTH

The study began by pairing mature males and females in the laboratory. After birth, the offspring were sexed, measured and individually marked. Body mass was measured with an electronic scale to the nearest 0·01 g and head width with a microscope to the nearest 0·1 mm. Within two days of birth, the litters were cross-fostered to exclude any confounding effects of possible variation in maternal quality, and manipulations of the rearing environment (i.e. offspring number) and body size at birth were performed. All the pups in a litter were replaced, resulting in litters with every pup originating from a different mother. Foster mothers' initial litter size (i.e. before manipulation) did not differ between manipulation groups (Pearson Chi-Square, $\chi^2 = 9{\cdot}31$, d.f. = 12, $P > 0{\cdot}6$). Moreover, the body size of foster mothers measured, as their postpartum body mass, did not differ between treatments (One-way ANOVA, $F_{3,28} = 0{\cdot}828$, $P > 0{\cdot}4$).

A 2 × 2 factorial experiment consisted of two treatments: manipulation of rearing environment (control: ± 0 pups and poor: + 2 pups) and manipulation of offspring body size (control and heavier pups). The manipulation of the rearing environment consisted of a foster mother's original litter being replaced with pups from donor mothers (whose litter sizes were the same as the foster's initial litter size) and two extra pups added. This manipulation is hereafter referred to as the 'poor' rearing environment. The same procedure was used for the 'control' rearing environment, but without the addition of extra pups. The manipulation of offspring size 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 mother. This manipulation is hereafter referred to as 'large' body size treatment. The same procedure was used for the 'normal' body size treatment but without either the increase in pup body mass or the reduction in the donor mothers' litter size. This method was based on the fact that, in small mammals, litter size and mean offspring body mass at birth are often negatively correlated (e.g. Kaufman & Kaufman 1987; this paper: $r = -0{\cdot}620$, $n = 171$, $P < 0{\cdot}001$) and thus, offspring in small litters are heavier than offspring in large litters. A combination of these treatments gave four manipulation groups: (i) normal body size in control rearing environment ($n = 17$), (ii) large body size in control rearing environment ($n = 17$), (iii) normal body size in poor rearing environment ($n = 16$), and (iv) large body size in poor rearing environment ($n = 17$).

MEASURES OF QUALITY

After manipulations in the laboratory, four females (one from each manipulation group) were placed in each enclosure. Breeding cages were placed near the corners of the enclosures (one in each corner) under rainproof covers and left open to allow mothers to move the pups into the enclosure. This method has been successful in our previous studies (Mappes *et al*. 1995; Koskela *et al*. 1998; Koskela, Mappes & Ylönen 1999). After all the offspring were weaned (*c*. 25 days from their birth), the offspring were measured and released back into the enclosures. The mothers, however, were returned to the laboratory. Body mass was measured with electronic scales and head width with a digital calliper. At the age of 45 days, the measurements of offspring body size were repeated, and the pups were returned to the laboratory. The second experimental run, which was identical to the first one except different females were used, subsequently started in the empty enclosures.

Offspring quality was determined from haematological measures, immunological parameters, parasitic infection and the proportional gain in body size from weaning to the age of 45 days. The proportional gain in size, calculated for both body mass and head width as (size at 45 days – size at 25 days)/size at 25 days, expresses the body mass (g) or head width (mm) gained during the independent life in relation to the size attained during the nursing period. The measure can therefore be used to estimate the ability of the offspring to compensate for the possible restrictions of maternal care as well as their capacity for growth in general.

HAEMATOLOGICAL AND IMMUNOLOGICAL MEASURES

Haematological measures and offspring immune function were assessed at the age of 45 days. Two retro-orbital blood samples were collected from each individual in heparinized capillary tubes (I.D. 0·5–0·6 mm). One of the capillary tubes was used for the white blood cell count (WBC) in a Bürker haemocytometer chamber (cells mL^{-1}). 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. The 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 serum IgG (U mL^{-1}). To measure a specific immune response, each individual was immunized with an intraperitoneal injection (0·1 mL) of bovine gamma globulin (BGG, Sigma Chemical Co, 200 μg) emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, MI). A total of 168 individuals were immunized immediately after the

Table 1. Offspring head width at birth (after manipulation), at weaning (25 days) and at the age of 45 days and the proportional gain in head width during the period from weaning to the age of 45 days in relation to the manipulations of rearing environment and body size at birth. (Nested ANOVA, foster mother (random effect) nested within environment and size manipulation and experimental runs (fixed effects))

Head width	Factor	d.f.	MS	*F*	*P*
Birth	Experimental run	1	1·570	4·678	0·034
	Environment	1	0·018	0·055	0·815
	Birth size	1	10·253	30·925	< 0·001
	Environment × size	1	0·015	0·045	0·832
	Foster	69	0·345	3·183	< 0·001
	Error	434	0·109		
25 days	Experimental run	1	2·593	6·484	0·014
	Environment	1	6·331	14·509	< 0·001
	Birth size	1	0·563	1·293	0·261
	Environment × size	1	0·118	0·268	0·607
	Foster	48	0·608	7·833	< 0·001
	Error	205	0·078		
45 days	Experimental run	1	0·149	0·493	0·485
	Environment	1	3·641	11·372	0·001
	Birth size	1	0·198	0·617	0·435
	Environment × size	1	1·476	4·558	0·037
	Foster	46	0·404	3·255	< 0·001
	Error	184	0·124		
Gain in head width	Experimental run	1	0·066	10·894	0·002
	Environment	1	0·002	0·288	0·594
	Birth size	1	< 0·001	0·005	0·942
	Environment × size	1	0·056	8·601	0·005
	Foster	46	0·008	4·028	< 0·001
	Error	183	0·002		

blood samples from individuals in the second experimental run were taken. At that time, individuals from the first run were *c*. 85 days old and those from the second run *c*. 45 days old. After immunization, all the animals were released back into the enclosures. Four weeks later (at age of *c*. 73 or 113 days), another blood sample was collected to determine the specific immune response to BGG. Response to BGG (anti-BGG antibodies) was also measured from the first blood sample to ensure that there was no previous exposure to the antigen or cross-reactive antibodies in the plasma. The four week period for mounting the response was determined from a previous laboratory experiment where the response of adult bank vole males to BGG was analysed 2, 4 and 6 weeks after immunization (E. Koskela, I. Jokinen, T. Mappes & T.A. Oksanen, unpublished data).

QUANTIFICATION OF ANTIBODIES

Plasma levels of total IgG and anti-BGG specific antibodies were determined by micro plate enzyme-linked immunosorbent assay (ELISA). Commercial antimouse IgG-specific antibodies were tested in order to find out whether they cross-react with bank vole immunoglobulin and could be used for assaying vole plasma samples. A strong cross-reactivity of these antibodies with vole immunoglobulin was found, and an ELISA method based on these antibodies was set up. First, the plates were coated either with BGG (10 μg mL^{-1}) in carbonate buffer (50 mmoles L^{-1}, pH 9·8) to determine anti-BGG specific antibody, or with antimouse IgG (M-8642, Sigma Chemical Co.) to quantify the total IgG level. After saturation with 1% bovine serum albumin (BSA, Roche Diagnostics, Germany) in phosphate buffered saline (PBS, pH 7·4), the samples were either diluted 1 : 100 (anti-BGG antibodies) or 1 : 50 000 (IgG) in 1% BSA/PBS, and incubated in wells. The bound bank vole immunoglobulin was then detected with antimouse IgG alkaline phosphatase conjugate (A-2179, Sigma Chemical Co.). Washing was performed between each step with PBS-Tween 20 (0·05%). P-nitrophenyl phosphate (1 mg mL^{-1}, Sigma Chemical Co.) was used as the substrate, and after the enzyme reaction the optical density was read with a Titertek plate reader (Flow Laboratories) at 405 nm. A pool of plasmas, collected from all immunized voles in the study, was used to calibrate the assay of anti BGG-specific antibodies. The concentration of anti-BGG in the pooled plasma was given 1000 artificial units per mL (U mL^{-1}) and the concentrations of samples were then expressed as U mL^{-1}. The assay of bank vole plasma IgG was calibrated similarly using a pool of 30 plasmas collected from voles in the study.

PARASITE INFECTION

The intensity of infection by *Eimeria* spp., genera of an intestinal protozoan (coccidian), was used to study the

effects of the manipulations on offspring quality. *Eimeria* spp. have endogenous developmental life cycles, and they are transmitted directly through ingestion of contaminated faeces. The endogenous stages develop in epithelial cells of the colon in bank voles (Lewis & Ball 1982). Because the parasites repeatedly penetrate and burst out of the epithelial cells at different stages of their life-cycle, they have the potential to cause severe harm to the host. Generally, symptoms such as diarrhoea, dehydration and weight loss are associated with heavy infection, which is referred to as coccidiosis (Yun, Lillehoj & Lillehoj 2000). *Eimeria* spp. have previously been found in bank vole populations in Finland (Laakkonen *et al.* 1998) and some species of *Eimeria* are known to have a negative effect on over-winter survival of small rodents (Fuller & Blaustein 1996). The faecal samples used to determine parasite infection were collected from voles at 45 days of age by individually placing the animals in small plastic containers for half an hour. The faeces (0·01–0·04 g) were stored in 2·5% aqueous potassium dichromate ($K_2Cr_2O_7$) to make 1 mL of suspension. The suspension was centrifuged for 3 min at 1750 r.p.m. 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 McMaster counting chamber, and the count was transformed into the number of *Eimeria* spp. oocysts per gram of the original sample.

DATA ANALYSIS

Variation in offspring body size, haematological measures, immune function and parasite infection (Tables 1–3) were analysed with nested ANOVA, where foster mother (random effect) was nested within offspring number and size manipulation and experimental run (fixed effects). Experimental run was included in all models in order to control for possible seasonal differences in conditions between the two experimental runs. The degrees of freedom were adjusted for unbalanced design by SPSS (version 10·1), which uses Satterthwaite's method. Sample size in all figures and tables is the number of individual offspring. As haematocrit and the gain in body size were measured as a proportion, an arcsine transformation was performed for these variables before analysis. Logarithmic transformations ($\log_{10}(x + 1)$) were performed on the values of anti-BGG antibody level, total serum IgG, white blood cell count and coccidian oocyst count to correct for the non-normality of the data.

Results

Offspring head width was significantly larger in offspring size manipulation groups after manipulation, but did not differ between the two rearing environments (Table 1, Fig. 1a). At the age of 25 days, offspring reared in poor environments had smaller head widths, whereas the effect of offspring size manipulation had disappeared (Table 1, Fig. 1b). At 45 days of age, there was a significant interaction between the rearing environment and size manipulation indicating that the growth of large individuals in poor environment had been retarded in comparison to the other groups (Table 1, Fig. 1c). A similar trend, although not as strong, could be detected in the measurements of body mass (Table 2, Fig. 2a–c). The proportional gain in body mass during the period from weaning to the age of 45 days was significantly higher for offspring reared in poor environments suggesting that offspring were trying to compensate for their smaller body size at weaning (Table 2, Fig. 3a). Moreover, there was an interaction between rearing environment and body size in the proportional gain in head width. The initially normal sized offspring reared in poor environments increased both their body mass and skeletal body size, whereas the initially large offspring had increased only their body mass (Table 1, Fig. 3b). However, the large offspring reared in control

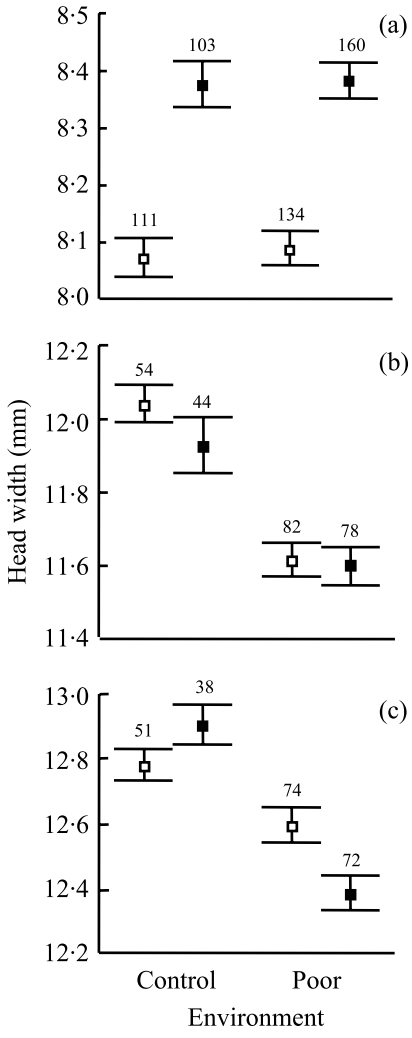

Fig. 1. Mean head width (± SE) in (a) at birth (after manipulation), (b) at weaning (25 days), and (c) at the age of 45 days in the different rearing environments (□, control group; ■, body size manipulation group). Sample sizes are given above the error bars.

Table 2. Offspring body mass at birth (after manipulation), at weaning (25 days) and at the age of 45 days and the proportional gain in body mass during the period from weaning to the age of 45 days in relation to the manipulations of rearing environment and body size at birth. (Nested ANOVA, foster mother (random effect) nested within environment and size manipulation and experimental runs (fixed effects))

Body mass	Factor	d.f.	MS	*F*	*P*
Birth	Experimental run	1	< 0·001	0·005	0·946
	Environment	1	0·099	1·120	0·293
	Birth size	1	3·362	38·066	< 0·001
	Environment × size	1	0·037	0·417	0·521
	Foster	69	0·092	3·942	< 0·001
	Error	434	0·023		
25 days	Experimental run	1	82·955	10·055	0·002
	Environment	1	139·784	15·524	< 0·001
	Birth size	1	6·258	0·696	0·408
	Environment × size	1	2·345	0·257	0·614
	Foster	48	12·550	7·871	< 0·001
	Error	205	1·594		
45 days	Experimental run	1	9·113	1·247	0·269
	Environment	1	27·887	3·745	0·058
	Birth size	1	1·028	0·137	0·712
	Environment × size	1	8·360	1·103	0·298
	Foster	45	10·091	4·958	< 0·001
	Error	165	2·035		
Gain in body mass	Experimental run	1	0·602	6·312	0·015
	Environment	1	1·090	11·126	0·002
	Birth size	1	< 0·001	< 0·001	0·998
	Environment × size	1	0·141	1·439	0·239
	Foster	43	0·128	5·369	< 0·001
	Error	159	0·024		

environments had gained more both in body mass and head width in comparison to the initially normal sized offspring (Tables 1 and 2, Fig. 3a,b).

The manipulations of rearing environment and offspring size at birth had a significant interaction in relation to plasma anti-BGG specific antibody level. Consistent with the proportional gain in body size, the initially large individuals reared in control environments and the initially normal sized individuals reared in poor environments had the highest antibody levels (Table 3, Fig. 4). The quantity of anti-BGG antibodies (U mL^{-1}) in the first blood samples was marginal in relation to the samples collected after immunization indicating that there was no previous exposure to the antigen (mean ± SE, first sample: 222·6 ± 9·9, n = 199, second sample: 6122·7 ± 1229·4, n = 148). The manipulations did not affect any other measures of haematology, immune function or parasite infection (Tables 3 and 4), and correlations between the measures were not found (Table 5). The prevalence of parasite infection in the whole data set was 71·3% (n = 202), and there was no significant difference in infection among the manipulation groups (Pearson Chi-Square, χ^2 = 2·530, d.f. = 3, P > 0·470).

Discussion

In many species, the decisions that parents make concerning the allocation of their reproductive effort between the number and size of their offspring, determine the most immediate rearing environment for the growing young. We studied the effects of the rearing environment (offspring number) on the quality of young bank voles with different initial body sizes at birth, to find out if the benefits of large body size at birth depend upon the quality of the rearing environment. The study design enabled a simultaneous manipulation of individual trait (body size) and selective environment (litter size), which has recently proved to be a fruitful approach to the study of life history evolution (Svensson & Sinervo 2000). As predicted, offspring body size at weaning (at the age of 25 days) was significantly lower in poor rearing environments. After a 3-week period of independent life (at the age of 45 days), the initially large individuals were larger or smaller in comparison to the initially normal sized individuals depending on whether they had been reared in control or poor rearing environments, respectively. Thus, large body size at birth seemed to be an advantage in 'normal' rearing environments, but a disadvantage in poor rearing environments. Analysis of proportional gain in body size during this period revealed that the initially large offspring reared in control environments had gained both in body mass and head width, even though their body size already was relatively large at weaning. The same was not true in poor rearing environments, the initially large offspring gained only in body mass, suggesting that they were

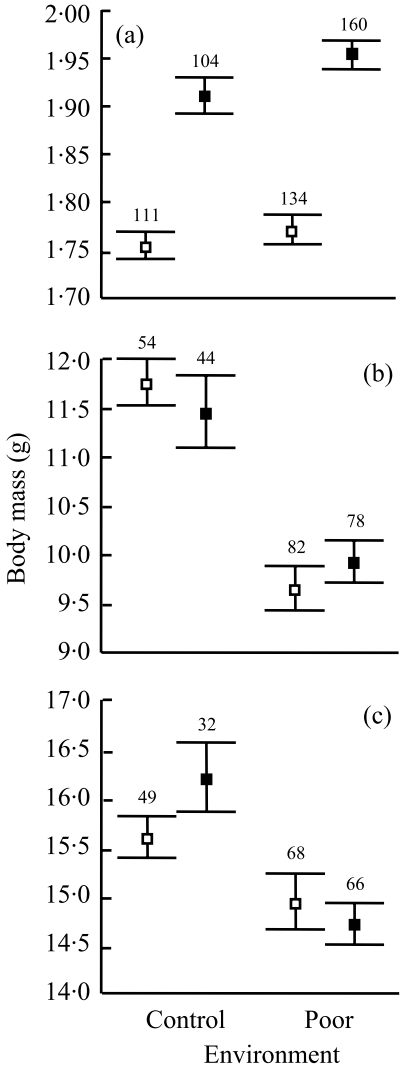

Fig. 2. Mean body mass (± SE) in (a) at birth (after manipulation), (b) at weaning (25 days), and (c) at the age of 45 days in the different rearing environments (□, control group; ■, body size manipulation group). Sample sizes are given above the error bars.

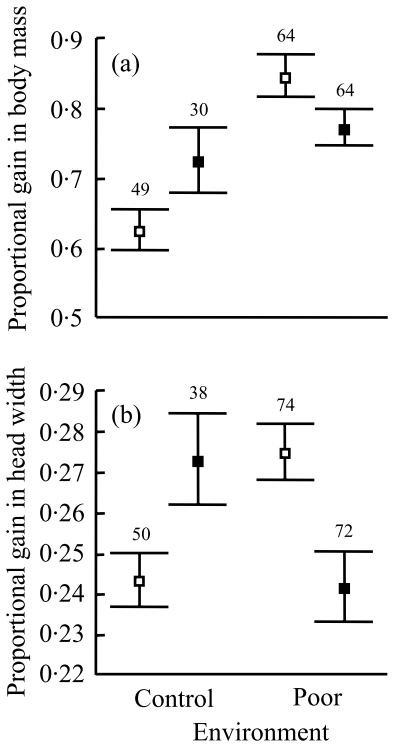

Fig. 3. Mean proportional gain (± SE) in (a) body mass and (b) head width in the different rearing environments from weaning (25 days) to the age of 45 days (□, control group; ■, body size manipulation group). Sample sizes are given above the error bars.

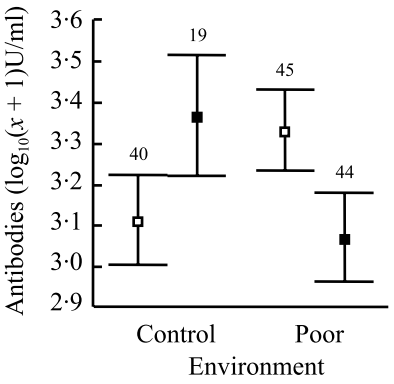

Fig. 4. Mean Anti-BGG specific antibody level (± SE) in the different rearing environments (□, control group; ■, body size manipulation group). Sample sizes are given above the error bars.

recovering from the poor conditions during their nursing period, but were not able to compensate for their smaller skeletal body size. The only group that showed actual compensatory growth (Hornick *et al*. 2000; Metcalfe & Monaghan 2001) were the initially normal sized offspring reared in poor environments, who were relatively small at weaning age, but afterwards gained both in body mass and head width.

These results suggest that individuals with a large body size at birth were able to maintain their body size during the nursing period and grow relatively fast after reaching independence from their mother, but only if the rearing environment remained the same. Moreover, in poor rearing environments, offspring were only able to compensate for their retarded growth during the nursing period if their body size at birth was normal. Being a large pup in a large litter was therefore the worst option, presumably because the reduced amount of resources, resulting from the increased number of siblings, was more costly for large pups compared to pups in the control body size group. The large body size at birth did not protect the offspring from the deleterious effects of the poor rearing environment, but instead reduced their chances of recovery. The considerable gain in body size by the initially large individuals reared in the control environment and initially normal sized individuals reared in the poor environment may denote their good overall condition, because a similar trend was detected in the anti-BGG specific antibody level with the fastest growing individuals having the highest antibody levels. With respect to the negative maternal

Table 3. Anti-BGG specific antibody level, plasma total IgG, haematocrit, blood white cell counts (WBC) and coccidian oocyst counts in relation to the manipulations of rearing environment and body size at birth. (Nested ANOVA, foster mother (random effect) nested within environment and size manipulation and experimental run (fixed effects))

	Factor	d.f.	MS	*F*	*P*
Anti-BGG antibody	Experimental run	1	0·104	0·188	0·666
	Environment	1	0·062	0·113	0·738
	Birth size	1	0·003	0·005	0·943
	Environment × size	1	2·180	3·921	0·052
	Foster	44	0·611	1·499	0·050
	Error	99	0·408		
IgG	Experimental run	1	0·030	0·377	0·541
	Environment	1	0·018	0·223	0·638
	Birth size	1	< 0·001	0·005	0·942
	Environment × size	1	0·209	2·520	0·117
	Foster	49	0·099	1·976	0·001
	Error	179	0·050		
Haematocrit	Experimental run	1	0·027	26·120	< 0·001
	Environment	1	0·003	2·454	0·121
	Birth size	1	< 0·001	0·617	0·434
	Environment × size	1	0·003	2·458	0·121
	Foster	47	0·001	1·156	0·249
	Error	175	< 0·001		
WBC	Experimental run	1	0·396	11·218	0·001
	Environment	1	0·019	0·542	0·463
	Birth size	1	< 0·001	< 0·001	0·999
	Environment × size	1	< 0·001	0·007	0·932
	Foster	46	0·035	0·956	0·558
	Error	181	0·036		
Coccidian oocyst count	Experimental run	1	8·969	2·806	0·098
	Environment	1	7·266	2·234	0·139
	Birth size	1	0·380	0·117	0·733
	Environment × size	1	4·221	1·291	0·260
	Foster	45	3·631	1·542	0·028
	Error	152	2·355		

Table 4. Descriptive statistics (mean ± SE (*n*)) for the original values of plasma total IgG (U mL^{-1}), white blood cell count (WBC, $\times 10^3$ cells mL^{-1}), haematocrit (%) and coccidian oocyst count ($\times 10^3$ oocysts g^{-1})

Environment:	Control		Poor	
Body size:	Control	Large	Control	Large
IgG	467 ± 55 (50)	503 ± 67 (37)	477 ± 51 (73)	416 ± 29 (73)
WBC	37 ± 3 (51)	36 ± 2 (36)	36 ± 2 (73)	37 ± 2 (72)
Haematocrit	48 ± 0·5 (50)	46 ± 0·5 (36)	47 ± 0·5 (71)	48 ± 0·4 (70)
Coccidian	10·6 ± 3·0 (42)	5·0 ± 2·1 (35)	7·3 ± 3·0 (68)	4·8 ± 1·3 (57)

effect hypothesis (Leamy 1981; Millar 1982), our results suggest, that the effects of prenatal maternal environment on offspring performance may be obscured by changes in the characteristics of the litter during the nursing period. Therefore, besides genes, environment and maternal effects, offspring performance may also be substantially affected by coincidental changes within the litter (e.g. offspring mortality).

The haematological measures of offspring quality and the number of Coccidian oocysts in offspring faeces did not reveal any differences among individuals with different body sizes at birth or reared in different environments. Earlier studies on blood parameters in *Clethrionomys* spp. reveal that haemoglobin levels vary relatively little between seasons and different populations (Sealander 1966; Kostelecka-Myrcha 1967). Moreover, these species have relatively high haemoglobin values and thus a better condition of gaseous exchange compared to other small mammals of the same body size, which may be the reason for their wide ranging geographical distribution. The infection of *Eimeri*a spp. either did not seem to cause severe harm to the animals, or our measures of condition and immune system were not sensitive enough to detect differences in infection. It is currently believed that cell-mediated immunity is the main factor conferring resistance to

Table 5. Pearson's product moment correlation coefficients between haematological measures, immune function and coccidian oocyst count. None of the correlations remain statistically significant after Bonferroni correction (Rice 1989)

		Anti-BGG	IgG	WBC	Haematocrit
IgG	*r*	0·039	–	–	–
	P	0·642	–	–	–
	n	146	–	–	–
WBC	*r*	–0·137	0·116	–	–
	P	0·099	0·080	–	–
	n	146	229	–	–
Haematocrit	*r*	–0·176	0·072	0·055	–
	P	0·037	0·284	0·407	–
	n	141	226	227	–
Coccidian oocyst count	*r*	–0·018	0·095	0·108	0·015
	P	0·838	0·185	0·131	0·836
	n	126	197	198	193

coccidiosis, although immune strategies against coccidia are extremely complex (Yun *et al*. 2000). We assessed serum IgG level and specific antibody response to BGG, which are measures of humoral immunity. Although response to BGG requires the cooperation of the antigen presenting cells, T and B cells, the two parameters measured may not be able to reflect all the changes in immune defence, e.g. against parasites on mucosal membranes of the gut.

In conclusion, a large body size at birth was advantageous in 'normal' rearing environments. This was revealed by the high quality of these individuals at the start of their independent life measured from their body size, capacity for further growth and specific antibody response to a novel antigen. In poor rearing environments, however, initially large individuals suffered from retarded growth, and as a consequence of their inability to compensate for the loss, did not catch up with the others in quality. This suggests that selection on offspring body size at birth is more likely to be context-dependent than strictly directional. The initially normal sized offspring in enlarged litters showed compensatory growth, both in their body mass and head width suggesting that a poor rearing environment alone did not reduce their quality. However, it is possible that the compensatory growth would have had some costs in the long term (Metcalfe & Monaghan 2001), although none were detected during the current study. Ultimately, the analyses confirmed the results from our previous study (Oksanen *et al*. 2001) suggesting that the quality of the offspring does not necessarily decrease with increasing offspring number. The benefits of large body size at birth were dependent on the quality of the rearing environment, i.e. offspring number, consistent with the negative correlation between the mean number and size of offspring observed in the original litters. The results therefore suggest that, in *C. glareolus*, offspring number and size are subject to correlational selection aimed at optimizing the combination of these two traits (Sinervo & Svensson 2002). Moreover, the effects of body size at birth and rearing environment on offspring quality interact, which indicates that besides the trade-off between offspring number and quality, maternal environmental effects may have the potential to influence the evolution of a mothers' allocation of reproductive effort between offspring number and size.

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