Journal of Animal Ecology

British Ecological Society

Journal of Animal Ecology 2012, **81**, 573–582 doi: 10.1111/j.1365-2656.2011.01937.x

Life in varying environments: experimental evidence for delayed effects of juvenile environment on adult life history

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Summary

1. The effects of environment experienced during early development on phenotype as an adult has started to gain vast amounts of interest in various taxa. Some evidence on long-term effects of juvenile environment is available, but replicated experimental studies in wild animals are still lacking.

2. Here we report the first replicated experiment in wild mammals which examines the long-term effects of juvenile and adult environments on individual fitness (reproduction, survival and health). The early development of bank vole (*Myodes glareolus*) individuals took place in either food-supplemented or un-supplemented outdoor enclosures. After the summer, adult individuals were reciprocally changed to either a similar or opposite resource environment to overwinter.

3. Adult environment had an overriding effect on reproductive success of females so that females overwintering in food-supplemented enclosures had a higher probability of breeding and advanced the initiation of breeding. However, the characteristics of their litters were determined by juvenile environment: females initially grown in food-supplemented conditions subsequently produced larger litters with bigger pups and a male-biased sex ratio.

4. In males, individuals growing in un-supplemented conditions had the highest survival irrespective of adult environment during winter, whereas in females, neither the juvenile nor adult environments affected their survival significantly. The physiological condition of voles in spring, as determined by haematological parameters, was also differentially affected by juvenile (plasma proteins and male testosterone) and adult (haematocrit) environments.

5. Our results suggest that (i) life-history trajectories of voles are not strictly specialized to a certain environment and (ii) the plastic life-history responses to present conditions can actually be caused by delayed effects of the juvenile environment. More generally, the results are important for understanding the mechanisms of delayed life-history effects as well as recognizing their population dynamic consequences.

Key-words: density dependence, fitness, mammals, maternal effects, *Myodes glareolus*, past and present environment, population cycles, predictive adaptive response, reciprocal transplantation

Introduction

Fitness components are regulated by interactive effects of an individual's genetic composition and its environment. Traditionally, environmental effects were understood only as the influence of present environmental conditions, but only quite recently, the concept of early growth conditions has received attention (reviewed in Lindström 1999; Monaghan 2008). Effects of the growing environment on the phenotype may act directly on the developing individual and/or indirectly through maternal effects. Maternal effects are controlled by both the mother's genetic composition and prevailing environmental conditions. Although their importance in shaping the phenotype of newborn individuals in various aspects of morphology and physiology is evident, it is less clear how well maternal effects show adaptive responses to match the phenotype to the present environment (Mousseau & Fox 1998).

Nutritional deficits during the growth period have been reported to result in a variety of morphological and

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physiological setbacks, for example reduced intellectual capacity (Fisher, Nager & Monaghan 2006), basal metabolic rate (Desai & Hales 1997), lipid metabolism (Lucas *et al.* 1996), glucose tolerance (Leger *et al.* 1997), immunity (Saino, Calza & Möller 1997), secondary sexual characters (Gustafsson, Qvarnström & Sheldon 1995) and success in gaining a good-quality territory (Verhulst, Perrins & Riddington 1997). Deficiencies in various characters at early life stages may result in poorer performance of an individual later on in life. Characteristics of the juvenile environment may, however, greatly differ from the environment experienced as an adult. This was recently demonstrated in an experiment conducted in a cichlid fish *Simochromis pleurospilus* (Taborsky 2006). In that study, the growth of adult fish was determined by current resource availability, but key reproductive traits like reproductive rate and offspring size were only influenced by juvenile growth conditions, irrespective of the ration received as adults.

In mammals, long-term studies of the effects of growing conditions are available mostly from the laboratory. For instance, daughters of mouse females that were food deprived during gestation gave birth to smaller litters (Meikle & Westberg 2001). The most elaborate proof for effects of juvenile environment in mammals has been provided by human studies (reviewed in Lummaa & Clutton-Brock 2002; Gluckman, Hanson & Spencer 2005; Rickard & Lummaa 2007). Conditions experienced during early development, both pre- and postnatal stages, have been shown to affect reproductive performance even over several generations (Bateson *et al.* 2004). In wild mammals, evidence for long-term effects of early-life conditions on reproductive success and survival is available in European rabbits (Rödel, von Holst & Kraus 2009) and red squirrels (Descamps *et al.* 2008). In *Myodes* voles, the potential effects of conditions experienced during prenatal and postnatal periods have been brought about already in the 1990s (Andreassen & Ims 1990; Ims 1990) and new studies are now slowly accumulating (see Oksanen *et al.* 2012). Together, these earlier studies indicate that early environment has the potential to influence fitness consequences in later life.

Environmentally induced changes could select for those individuals which would be better adapted to prevailing environmental conditions. Such adaptive responses can include short-term changes in physiology and behaviour, as well as include long-term adjustments to conditions predicted by the state of the environment when the organism is in its early stages of growth (Monaghan 2008). If such specialized types existed, changing the environmental conditions during an individual's life span should result in a negative effect on its fitness. In other words, individuals specialized to, for example, good-quality environment should perform worse in relation to the other genotypes when moved to a poor-quality environment and *vice versa* (Mills *et al.* 2007a).

We constructed an experimental set-up in outdoor enclosures aimed at unravelling the effects of juvenile and adult environmental conditions on reproduction, survival and health in the bank vole (*Myodes glareolus* Schreber 1780). As the environmental variable, we decided to manipulate food resources, as food is one of the key ecological factors determining life-history evolution in this species (Koskela *et al.* 1998; Prevot-Julliard *et al.* 1999; Eccard & Ylönen 2001). This is not only caused by seasonal changes in food abundance, but also by multiannual fluctuations in vole densities (e.g. Korpimäki *et al.* 2005; Kallio *et al.* 2009). Food also has direct density-dependent effects on physiological health of individuals and population growth rate (Huitu *et al.* 2003, 2007). In our experiment, the early development of bank voles took place during the summer in either food-supplemented or un-supplemented (control) enclosures. After summer, the treatment of half of the individuals was changed, while the other half overwintered in the same conditions in which they were grown (Fig. 1). The survival, reproduction and health of individuals were monitored at the beginning of the next breeding season in May. The objective of our study was to identify the role of juvenile and adult environments on fitness traits as an adult. These fitness traits include breeding characteristics (breeding time and probability, litter size, offspring body weight, litter sex ratio) of females and life-history characteristics of over-wintered individuals (survival, weight, condition and haematological characteristics). In addition to the main effects, we tested whether the effects of these two environments are independent or correlated (i.e. strengthen each other) and whether reciprocal change in resource environment would give support for the life-history traits being highly plastic or specialized in a particular juvenile environment. Based on earlier studies, we predicted the breeding characteristics to be dictated by juvenile environment and adult characteristics by adult environment. We further predicted that the individuals would be specialized in a certain juvenile environment, so that a change in environment from juvenile to adulthood would result in a setback.

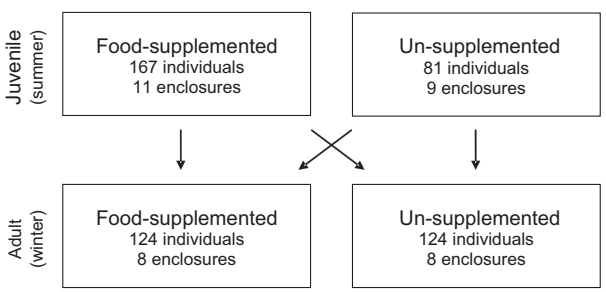

Fig. 1. The study design involved two parts: manipulation (by food supplementation) of juvenile environment of bank voles during summer (breeding season) and manipulation of adult environment during the following winter. After summer, study individuals were reciprocally transplanted into same or opposite adult environment. There were four treatment groups: (i) changing from food-supplemented juvenile environment to un-supplemented adult environment, (ii) changing from un-supplemented juvenile environment to food-supplemented adult environment, (iii) staying in un-supplemented environment and (iv) staying in food-supplemented environment.

Materials and methods

STUDY SPECIES AND SITE

The bank vole is a widespread and common small rodent in northern Europe, which inhabits most available habitats (Stenseth 1985), but is mainly a resident of open or semi-open areas such as old fields and forest edges. Bank vole populations in northern Europe typically fluctuate in a cyclic manner with very high population peaks every 3–4 years followed by steep declines, or crashes, in population density (Korpimäki *et al.* 2005; Kallio *et al.* 2009). However, the previously regular cyclic population changes have more or less faded in several regions, climate change being one potential cause behind this phenomenon (Ims, Henden & Killengreen 2008). Bank voles feed mostly on plants, especially seeds (Hansson 1979). In our study area in central Finland, bank voles breed promiscuously during summer months (from May to September) producing up to four litters, each containing 1–10 offspring (Koivula *et al.* 2003; Mappes & Koskela 2004; Mills *et al.* 2007b). The first cohorts of the summer reproduce already during their first summer (Mappes, Ylönen & Viitala 1995; Koivula *et al.* 2003). Reproductive success and survival of female bank voles is determined by ecological factors: food resources and population density (Koskela *et al.* 1998; Koskela, Mappes & Ylönen 1999). Mating success of males is determined by male–male competition (Oksanen *et al.* 1999) and modified by the trade-off between male testosterone level and immunocompetence (Mills *et al.* 2009, 2010; Schroderus *et al.* 2010).

The study was conducted in outdoor enclosures located in Konnevesi, Central Finland (62°37′N, 26°14′E). The enclosures are constructed of 1·25-m high sheet metal extending 0·5 m underground. Each enclosure covers 2000 square metres of old farmland presently growing several species of grass and herbs (see details in the study by Oksanen, Koskela & Mappes 2002). In every enclosure, there are 20 permanent trapping sites distributed evenly in the enclosure as a four by five grid. Every trapping site consists of a sheet-metal chimney housing a multiple-capture 'Ugglan Special' trap baited with sunflower seeds and potatoes when trapping. Between the trappings, traps were empty and not set, thus allowing voles free entry and exit enabling efficient trapping.

STUDY DESIGN

The study design involved two parts such as manipulation of juvenile environment of bank voles during summer (breeding season) and manipulation of adult environment during the following winter. Study populations were founded by releasing four males and four females originating from second generation laboratory stock from wild ancestors in 20 enclosures in June. These founder individuals reproduced freely until the end of the breeding season. As a manipulation of environment, half of the vole populations were continuously supplementary fed (good-quality environment), whereas the other half were left un-supplemented (control environment). Supplementary feeding was conducted by offering sunflower seeds *ad libitum* at every trap site in the enclosure. The F1 generation offspring that were born and grown in these populations formed the study individuals for the experiment. Altogether 248 F1 individuals were trapped from the enclosures in August, 167 from food-supplemented and 81 from un-supplemented environment. F1 individuals were distinguished by body weight and reproductive state. All females entering the experiment had not bred during their first summer.

Individuals were brought to laboratory in the beginning of October for 3–7 days where they were measured. Head width (a proxy of structural size) was measured to the nearest tenth of a millimetre with a digital calliper and weighed to the nearest 0·1 g by a digital scale. The condition was calculated as standardized residuals from the linear ordinary least squares regression of body mass on head width (Schulte-Hostedde *et al.* 2005). Right after measurements, animals were assigned to 16 enclosures for the second part of the experiment (manipulation of adult environment during winter, Fig. 1) and released to the enclosures in the middle of October.

There were four treatment groups: (i) changing from food-supplemented juvenile environment to un-supplemented adult environment, (ii) changing from un-supplemented juvenile environment to food-supplemented adult environment, (iii) staying in un-supplemented environment and (iv) staying in food-supplemented environment (Fig. 1). Supplementary feeding during winter was conducted in a similar fashion as in the summer. All the individuals were released to a different enclosure than where they had grown during summer. Every enclosure included randomly chosen 10–11 individuals from food-supplemented environment and 5–6 individuals from un-supplemented growing environment, and sexes were assigned equally to different enclosures. Morphological characteristics of individuals did not differ between the winter treatment groups (ANOVA, all $P > 0{\cdot}188$).

After winter, in the beginning of next breeding season in May, all survived individuals were trapped from the enclosures and brought to the laboratory where they were blood sampled, measured and possible breeding in females was observed. Females were checked for signs of lactation revealing reproduction before May. Sex of newborn pups was determined by the help of visual cues and the length of anogenital distance (Koskela *et al.* 2009). Sex ratio was calculated as the proportion of male pups in a litter. Date of parturition is defined as the date of May.

ANALYSIS OF PLASMA PROTEINS, IMMUNOCOMPETENCE AND TESTOSTERONE

As a proxy of the health state and physiological condition of vole individuals, the following haematological indices were measured: plasma protein content, total immunoglobulin G level (IgG), haematocrit and male testosterone (T) level. We fully recognize that these measures only represent some components of overall physiological condition of an individual. For instance, several arms of the vertebrate immune system should be studied for a fuller understanding of immunocompetence. However, the measures we use here are known to be affected by environmental conditions (cycle phases) and to correlate with performance of voles (Huitu *et al.* 2007; Mills *et al.* 2010). Three blood samples (1×18 and 2×75 μL) were collected from the retro-orbital vein by heparinized capillary tubes. Samples were centrifuged at 12 000 *g* for 5 min to separate blood plasma from blood cells. After centrifuging, haematocrit was measured as the ratio of red blood cells to the whole blood volume by measuring the length of the two columns. Plasma was thereafter frozen in capillaries at −20 °C for later analyses of plasma proteins, immunocompetence and testosterone.

Plasma protein concentration (mg mL^{-1}) was measured by the Bradford method. A microplate modification of BioRad protein assay (Bio-Rad Laboratories Inc., Hercules, CA, USA) was used with bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO, USA) as the calibrating protein.

Total immunoglobulin G level (IgG) in plasma was measured by microplate enzyme-linked immunosorbent assay (ELISA). Commercial anti-mouse IgG-specific antibodies were used for the analysis (described in full detail by Oksanen *et al.* 2003). Vole plasma samples

were added to plates coated with anti-mouse IgG, and bound immunoglobulin was detected with anti-mouse IgG alkaline phosphatase conjugate (A-21798; Sigma Chemical). *P*-nitrophenyl phosphate (1 mg mL^{-1}; Sigma Chemical) was used as the substrate, and after the enzyme reaction, optical density was read at 405 nm. Measured concentrations were calibrated against a pool of vole plasmas which was given a concentration of a 1000 artificial units per mililitre.

Plasma testosterone level of males was measured using a radioimmunoassay kit (TESTO-CTK, DiaSorin; Byk-Sangtec Diagnostica GmbH & Co, Dietzenbach, Germany). Fifty microlitre of each blood sample was competed for 3 h at 37 °C with 500 μL of ^{125}I-labelled testosterone for antibody-binding sites in tubes coated with a T antiserum. The amount of testosterone in samples is inversely related to the amount of radioactivity. Testosterone concentration is then determined by extrapolation from the standard calibration curve (described in full detail in Mills *et al.* 2007b).

DATA ANALYSIS

Binary (survival and probability of breeding) and binomial (sex ratio) response variables were analysed using generalized linear mixed models (PROC GLIMMIX) in SAS® v. 9.1 (SAS Institute Inc., Cary, NC, USA) with binary/binomial distribution, logit link function and Satterthwaite calculations of degrees of freedom. Analyses of continuous variables were performed in SPSS® v. 15.0 (IBM, Armonk, NY, USA) using generalized linear mixed models with Satterthwaite calculations of degrees of freedom. Treatments (manipulation of juvenile and adult environment: food-supplemented vs. un-supplemented) were entered in all the models as fixed factors and enclosure as a random factor. As the weight and condition of individuals were affected by juvenile environment (Table 1), weight in October was used as a covariate in the analyses. When analysing characteristics of pups, mother identity nested within enclosure was used as a random factor to control for litter effects. In pup weight analysis litter size was included as a covariate. In analysis of female weight and condition, breeding status of females was used as a covariate. Each analysis was started from a model that included all the above-mentioned factors as main effects and their two-way interactions. The model was then hierarchically simplified by dropping the nonsignificant ($P > 0{\cdot}05$) main effects and interactions one by one.

Results

REPRODUCTION

When the effects of two environments (juvenile and adult) were analysed, we found that the adult environment (overwintering conditions) affected significantly the probability and starting of breeding. Out of in total 77 females surviving over winter, 38 were observed breeding in May, whereas 39 did not breed (Table 2). Probability of breeding was higher among females in the food-supplemented overwintering environment (Table 3); out of the 41 females receiving supplementary feeding in winter, 28 (68%) reproduced, whereas of the 36 un-supplemented females, only 10 (28%) reproduced. Females overwintering in a food-supplemented environment started breeding earlier than females in an un-supplemented environment (Fig. 2a, Table 3). In addition, 14 females were found to have bred already at least once before the trapping (and reproduction) in May by signs of lactation, all of them belonging to the food-supplemented treatment in winter.

Table 1. Descriptives [mean ± SE (*n*)] and GLMM analyses of the characteristics of individuals at the start of overwintering part of the experiment in October. Nonsignificant interaction terms were removed, and enclosure is included as a random factor in the models. Numerator d.f. = 1 in both models. Intercept corresponds to a male in food-supplemented juvenile environment

	Juvenile environment	
	Food-supplemented	Un-supplemented
Males		
Weight	17·45 ± 0·37 (82)	16·25 ± 0·30 (40)
Condition	0·22 ± 0·13 (82)	−0·15 ± 0·12 (40)
Females		
Weight	15·81 ± 0·31 (85)	17·01 ± 0·42 (41)
Condition	−0·26 ± 0·10 (85)	0·23 ± 0·12 (41)

Factor	Estimate ± SE	DDF	*F*	*P*
Weight				
Intercept	17·268 ± 0·496			
Sex	−1·460 ± 0·420	243·02	1·80	0·181
Juvenile environment	−0·698 ± 0·795	13·64	0·13	0·728
Sex × Juvenile environment	1·906 ± 0·757	243·02	6·34	0·012
Condition				
Intercept	0·191 ± 0·153			
Sex	−0·434 ± 0·146	244·0	0·15	0·701
Juvenile environment	−0·252 ± 0·249	15·49	0·36	0·555
Sex × Juvenile environment	0·766 ± 0·262	244·0	8·52	0·004

Instead, the juvenile environment affected the characteristics of breeding: litter size and weight of individual pups corrected by litter size and offspring sex ratio. Females grown in food-supplemented environment gave birth to larger litters with heavier individual pups and a male-biased sex ratio (Fig. 2b, Tables 2 and 3). Importantly, the manipulations of the two environments had no significant interactive effects on breeding characters (all interaction terms between juvenile and adult environments $P > 0{\cdot}23$).

Population growth during summer was faster in supplementary-fed populations which ended up being larger than unfed populations ($t = -5{\cdot}674$, d.f. = 18, $P < 0{\cdot}001$). Population sizes in supplementary-fed and unfed summer populations in October were (average ± SE) 50·9 ± 4·8 and 16·4 ± 3·3, respectively. After winter, population sizes of supplementary-fed (13·4 ± 3·3) and unfed populations (8·6 ± 1·4) did not differ ($t = -1{\cdot}269$, d.f. = 13, $P = 0{\cdot}227$).

SURVIVAL

A total of 49 of 112 (44%) males and 77 of 118 (65%) females survived over the winter. Females survived significantly better than males ($F_{1,225} = 8{\cdot}72$, $P = 0{\cdot}004$), and therefore,

Table 2. Descriptives of breeding characteristics [mean ± SE (*n*)] of females and haematological characteristics in May

Juvenile env.	Food-supplemented		Un-supplemented	
Adult env.	Food-supplemented	Un-supplemented	Food-supplemented	Un-supplemented
Breeding time	11·44 ± 1·24 (18)	16·67 ± 0·49 (6)	13·56 ± 1·73 (9)	18·00 ± 1·52 (3)
% breeding	0·64 ± 0·09 (28)	0·28 ± 0·09 (25)	0·77 ± 0·12 (13)	0·27 ± 0·14 (11)
Litter size	6·06 ± 0·27 (18)	5·00 ± 0·31 (7)	4·90 ± 0·62 (10)	4·00 ± 1·16 (3)
Pup weight	1·78 ± 0·02 (109)	1·87 ± 0·03 (29)	1·67 ± 0·03 (43)	1·81 ± 0·06 (12)
Sex ratio	0·535 ± 0·059 (18)	0·475 ± 0·091 (6)	0·307 ± 0·081 (9)	0·5 ± 0 (3)
Testosterone	1·45 ± 0·30 (11)	0·79 ± 0·22 (11)	3·72 ± 1·10 (8)	1·45 ± 0·47 (9)
Plasma proteins	42·57 ± 2·91 (23)	40·64 ± 2·56 (27)	35·84 ± 2·97 (13)	33·65 ± 3·06 (17)
Males	36·03 ± 6·24 (6)	37·78 ± 4·18 (10)	35·70 ± 8·19 (4)	28·24 ± 4·09 (8)
Females	44·88 ± 3·19 (17)	42·32 ± 3·27 (17)	35·90 ± 2·82 (9)	38·46 ± 4·04 (9)
IgG	1190·57 ± 101·98 (35)	914·91 ± 71·77 (33)	1244·74 ± 133·40 (19)	1088·32 ± 72·59 (19)
Males	1046·82 ± 92·86 (11)	717·18 ± 45·70 (11)	1050·00 ± 209·41 (8)	1070·56 ± 93·49 (9)
Females	1256·46 ± 141·78 (24)	1013·77 ± 99·39 (22)	1386·36 ± 168·04 (11)	1104·30 ± 113·90 (10)
Haematocrit	52·32 ± 0·71 (35)	50·91 ± 0·63 (33)	52·58 ± 0·92 (19)	51·37 ± 0·65 (19)
Males	54·55 ± 0·97 (11)	51·09 ± 0·88 (11)	55·00 ± 1·23 (8)	52·22 ± 0·68 (9)
Females	51·29 ± 0·87 (24)	50·82 ± 0·85 (22)	50·82 ± 1·06 (11)	50·60 ± 1·05 (10)

Table 3. GLMM table of breeding characteristics of females in May. Numerator d.f. = 1 in all models. Nonsignificant interaction terms were removed, and enclosure is included as a random factor in the models. Intercept corresponds to an individual in food-supplemented juvenile and adult environment

Response variable	Factor	Estimate ± SE	DDF	*F*	*P*
Breeding time	Intercept	11·512 ± 1·047			
	Adult environment	4·962 ± 1·785	10·442	7·723	0·019
	Juvenile environment	1·916 ± 1·638	29·53	1·367	0·252
Probability of breeding	Intercept	0·580 ± 0·611			
	Adult environment	−1·969 ± 0·883	11·08	4·97	0·047
	Juvenile environment	0·540 ± 0·594	74	0·83	0·366
Litter size	Intercept	6·055 ± 0·356			
	Adult environment	−1·037 ± 0·584	9·559	3·158	0·107
	Juvenile environment	−1·113 ± 0·473	32·905	5·547	0·025
Pup weight	Intercept	2·204 ± 0·115			
	Adult environment	0·029 ± 0·059	34·029	0·245	0·624
	Juvenile environment	−0·122 ± 0·054	32·182	5·017	0·032
	Litter size	−0·070 ± 0·018	36·522	14·791	< 0·001
Sex ratio	Intercept	0·199 ± 0·272			
	Adult environment	−0·021 ± 0·480	11·49	0·00	0·965
	Juvenile environment	−0·732 ± 0·339	33	4·67	0·038

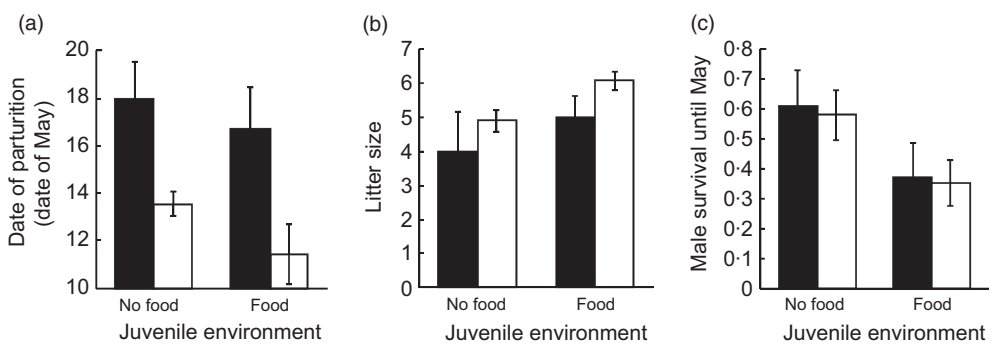

Fig. 2. (a) Females in a food-supplemented adult environment breed earlier (date of May, mean ± SE) compared with females in un-supplemented adult environment. (b) Females from a food-supplemented juvenile environment produce larger litters (mean ± SE) compared with females from un-supplemented juvenile environment. (c) Male survival over winter (mean ± SE) is reduced in individuals receiving food supplementation in juvenile environment. Open bars denote food supplementation in adult environment and filled bars un-supplemented adult environment.

sexes were analysed separately in further analyses of survival. A food-supplemented juvenile (growing) environment reduced male survival as compared to those grown in an un-supplemented environment (Fig. 2c), whereas in females, the treatments had no effect on survival (Table 4). Again, the manipulations of the two environments had no significant interactive effects on survival of individuals (all interaction terms between juvenile and adult environments $P > 0{\cdot}68$).

MORPHOLOGICAL AND HAEMATOLOGICAL VARIABLES

The body mass of females in May was higher for breeding individuals. Also food supplementation during winter and in the preceding summer increased female body mass (Table 4). The condition of females was enhanced by both breeding and food supplementation during winter. No effects were found on body mass and condition of males, but their testosterone level was negatively associated with food supplementation in the juvenile environment in previous summer (Tables 4 and 5). The concentration of plasma proteins was higher for individuals receiving supplemental food as juveniles, whereas adult environment had no effect (Fig. 3a, Table 5). Total immunoglobulin G level (IgG) was not significantly affected by the treatments, but females showed higher values than males (Fig. 3b, Table 5). Haematocrit level was increased by supplemental food during winter, but this effect was observed only in males (Fig. 3c, Table 5). The manipulations of the two environments did not have any significant interactive effects on morphological and haematological variables of individuals (all interaction terms between juvenile and adult environments $P > 0{\cdot}14$).

Discussion

The results from our long-term experiment conducted in enclosed small mammal populations support the idea (Lindström 1999) that the fitness of individuals is not only determined by their present environment but also by the growing environment in the early stages of an individual's life. According to the results, both juvenile and adult environments had significant sex-dependent effects on reproduction, survival and physiology in bank voles. In females, the overwintering environment experienced as an adult affected their breeding success as food supplementation enhanced the probability of breeding and advanced the initiation of reproduction. However, fitness of females was also determined by the conditions they experienced as juveniles, as food supplementation in the past increased their litter size and offspring weight corrected by litter size as well as biased the sex-ratio of their litters towards males. Probability of male survival to spring was quite surprisingly the highest for individuals whose juvenile growth environment was not manipulated by food supplementation. This finding is supported by the result that males from these un-manipulated juvenile growing conditions had also better breeding condition in the onset of the next breeding season as indicated by their higher testosterone levels. Together, these results provide one of the first empirical evidence of lifelong effects of early growth

Table 4. GLMM table of survival until May and weight and condition in May. Numerator d.f. = 1 in all models. Nonsignificant interaction terms were removed, and enclosure is included as a random factor in the models. Intercept corresponds to an individual in food-supplemented juvenile and adult environment

Response variable	Factor	Estimate ± SE	DDF	*F*	*P*
Females					
Survival	Intercept	0·561 ± 1·455			
	Adult environment	−0·118 ± 0·869	10·54	0·02	0·894
	Juvenile environment	−0·119 ± 0·483	114	0·06	0·806
	Weight in October	0·015 ± 0·084	114	0·03	0·855
Weight	Intercept	24·046 ± 0·658			
	Adult environment	−2·927 ± 0·765	9·475	14·633	0·004
	Juvenile environment	−1·118 ± 0·568	60·704	3·873	0·054
	Breeding	2·056 ± 0·625	68·670	10·813	0·002
Condition	Intercept	0·121 ± 0·211			
	Adult environment	−1·009 ± 0·232	10·389	18·909	0·001
	Juvenile environment	−0·026 ± 0·201	63·269	0·017	0·896
	Breeding	0·763 ± 0·213	60·720	12·913	0·001
Males					
Survival	Intercept	2·047 ± 1·514			
	Adult environment	0·197 ± 0·580	12·21	0·12	0·740
	Juvenile environment	0·886 ± 0·432	108	4·20	0·043
	Weight in October	−0·160 ± 0·087	108	3·35	0·070
Weight	Intercept	24·611 ± 0·682			
	Adult environment	−0·126 ± 0·843	12·106	0·022	0·884
	Juvenile environment	−0·681 ± 0·805	42·341	0·716	0·402
Condition	Intercept	0·0386 ± 0·205			
	Adult environment	−0·259 ± 0·262	13·081	0·971	0·342
	Juvenile environment	0·102 ± 0·219	41·300	0·215	0·645

Table 5. GLMM table of haematological characteristics in May. Numerator d.f. = 1 in all models. Nonsignificant interaction terms were removed, and enclosure is included as a random factor in the models. Intercept corresponds to a male in food-supplemented juvenile and adult environment

Response variable	Factor	Estimate ± SE	DDF	*F*	*P*
Plasma proteins	Intercept	37·57 ± 3·87			
	Adult environment	−0·83 ± 4·48	10·761	0·035	0·856
	Juvenile environment	−6·72 ± 2·64	66·777	6·467	0·013
	Sex	6·41 ± 2·69	65·541	5·674	0·020
IgG	Intercept	1012·82 ± 108·44			
	Adult environment	−222·41 ± 110·68	11·028	4·038	0·070
	Juvenile environment	143·91 ± 98·57	95·208	2·131	0·148
	Sex	236·20 ± 98·39	97·527	5·763	0·018
Testosterone	Intercept	1·857 ± 0·522			
	Adult environment	−1·311 ± 0·667	10·689	3·865	0·076
	Juvenile environment	1·326 ± 0·523	32·269	6·426	0·016
Haematocrit	Intercept	54·691 ± 0·874			
	Adult environment	−3·140 ± 1·143	101·000	6·039	0·016
	Juvenile environment	0·110 ± 0·728	101·000	0·023	0·880
	Sex	−3·582 ± 1·019	101·000	9·302	0·003
	Sex × Adult environment	2·747 ± 1·438	101·000	3·653	0·059

conditions on key life-history traits in mammals in a natural habitat.

The various haematological characteristics give an interesting insight into how direct and delayed environmental effects affect the phenotype of individual voles as adults. Previous studies in the bank vole show that the testosterone level of an individual is of great importance in the reproductive success of males (Mills *et al*. 2007b, 2009). Here, interestingly, testosterone levels of males were significantly lower in males, which were grown in good environment and food supplementation in adult environment, at the time of maturation, failed to increase testosterone levels significantly (Tables 2 and 5). Whether the lower testosterone levels appeared costly for males remains unclear as male reproductive success could not be detected in this study. Furthermore, the optimal testosterone level is tricky to interpret as high testosterone levels are clearly costly, for example for immune response of bank vole males (Mills *et al*. 2009, 2010). Still, our study suggests that when studying the factors affecting maturation and reproductive success of small mammal males, also the delayed effects of growing environment should be taken into account whenever possible (Mills *et al*. 2007a). Instead, the finding that high haematocrit was affected only by the adult environment is easier to explain because haematocrit is very much a measure of an individual's present condition and nutritional status (Ots, Murumägi & Hõrak 1998; Potti *et al*. 1999). Plasma proteins are also considered as a measure of individual condition at present (Ots, Murumägi & Hõrak 1998; Johnson 1999; Fuhrman, Charney & Mueller 2004), but interestingly, it was here affected only by the juvenile environment so that the individuals growing with supplemental food had significantly higher plasma protein levels as adults (Fig. 3a, Table 5). Concerning total immunoglobulin G level (IgG), the only significant effect found was that females showed higher IgG levels. This difference is not necessarily easy to interpret, because the expression of varying IgG levels is two-sided: an individual with a high concentration of IgG in the plasma can either have a high level of immune defence or be currently fighting an infection (Ots, Murumägi & Hõrak 1998; Adamo 2004).

Experimental studies where the importance of present environment is compared to the past one in the field are still scarce in the literature. One of the most comprehensive studies was conducted by Ergon, Lambin & Stenseth (2001a), where the authors transplanted field vole individuals before the breeding season between sites in which voles differed in average overwintering body mass. They discovered that individuals adjusted their midwinter body size and spring growth rate as well as onset of reproduction not to the past but according to the present environment into which they were transplanted. This led the authors to conclude that the immediate environment has an overriding role in shaping life-history traits of small rodents, and further, that there is no need to invoke intrinsic mechanisms in the study of life-history variation within vole populations (Ergon, Lambin & Stenseth 2001a). However, as their study concentrated basically only on two traits, timing of maturation and body mass, this conclusion may be premature. Our aim was to measure the central life-history traits of individuals by monitoring their long-term survival and onset of reproduction in enclosed field populations and recording their physiological condition and characteristics of breeding at the start of the breeding season. Here we suggest that the intrinsic effects may have a role in shaping life-history traits of small rodents, as early individual history had quite dramatic effects on adult phenotypes irrespective of adult environment.

Bank voles, as small rodent populations in general, show both seasonal and multiannual cyclic fluctuations in population density (Korpimäki *et al*. 2005; Kallio *et al*. 2009), and individual voles therefore face density-related changes (e.g. food resources, pathogen pressure) in their environment during their life (e.g. Soveri *et al*. 2000; Huitu *et al*. 2003, 2007). This kind of predictable environment could select for individuals capable of programming their development early in life

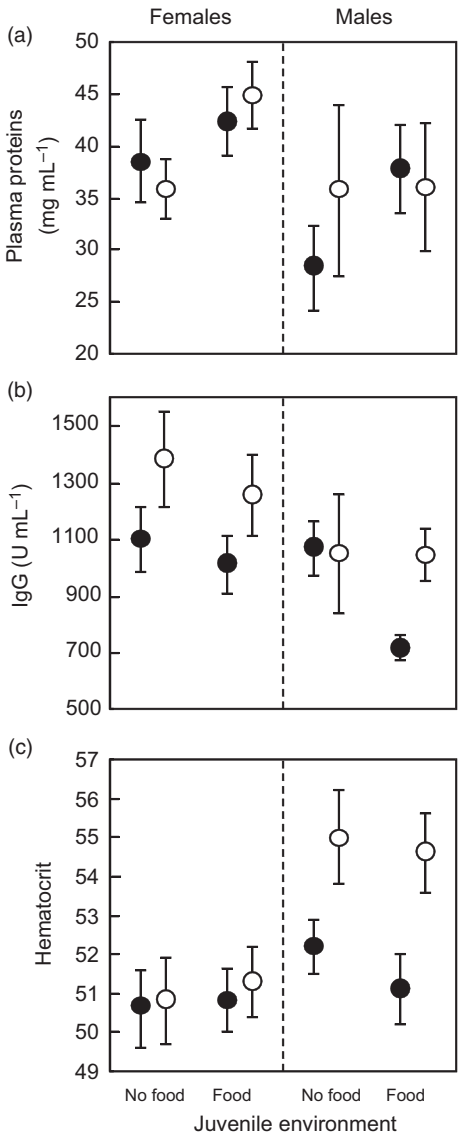

Fig. 3. (a) Plasma protein content (mg mL^{-1}, mean ± SE) is higher among individuals receiving food supplementation in juvenile environment and higher among females. (b) IgG (U mL^{-1}, mean ± SE) is higher among females and tends to be higher among individuals receiving food supplementation in adult environment. (c) Individuals in food-supplemented adult environment have higher haematocrit levels in May (mean ± SE) compared with individuals in un-supplemented adult environment. Males also have higher haematocrit compared with females. Open circles denote food supplementation in adult environment and filled circles un-supplemented adult environment.

to fully utilize environmental conditions as adults as predicted by the predictive adaptive response (PAR) hypothesis (Gluckman, Hanson & Spencer 2005; Rickard & Lummaa 2007). However, here the survival or reproductive success of individuals was only directly affected by present environment or delayed-affected by the past, and switching between juvenile and adult environments did not cause any significant changes in life-history trajectories of voles, that is an individual grown in poor environment did not suffer if it was switched to rich adult environment and *vice versa*. Consequently, the present study does not support the idea that vole individuals were strictly specialized to certain environment during their early life. The early growing environment seems to constrain certain life-history traits, whereas others are still plastic to respond directly to the present environmental conditions.

Delayed life-history effects, such as found in the present study, are also of great interest because of their potential population dynamic consequences. Recently, Beckerman *et al.* (2002) reviewed the current knowledge on this topic and recognized that delayed life-history effects might be a factor leading to delayed density dependence in population dynamics. In the present case, this could arise as a function of cohort effects, where the future performance of a group of individuals is affected synchronously by the quality of their growing environment. Here, the traits responding in a delayed fashion included central factors of female reproductive effort: the number and weight of pups as well as litter sex ratio. These traits are not only important fitness components of individual females, but they may also affect population growth rate. Consequently, as a delayed density-dependent structure is a necessity for vole cycles to exist (Turchin 2003), the present findings indicate a possible intrinsic factor influencing population dynamics (Yoccoz *et al.* 2001; Huitu *et al.* 2003, 2007).

Conclusions

Our experiment reveals intriguing evidence of delayed effects of environmental conditions experienced in the past on life-history trajectories of individuals. Consequently, in contrast to earlier suggestions (Ergon, Lambin & Stenseth 2001a; Ergon *et al.* 2001b), we show evidence that memory of past conditions may lead to delayed effects on life-history traits. Obviously, here we cannot separate whether delayed effects are mediated directly by availability of food resources or some other covarying factor, such as density and intraspecific competition. Similarly, the design of our study compares two different stages of the life cycle of bank voles (juvenile vs. adult), when unavoidably also many other things change, including season (breeding vs. nonbreeding). Still, we conclude that it is necessary to take into account intrinsic mechanisms when explaining the conspicuous variation in life-history traits within vole populations (see also Oksanen *et al.* 2012). Moreover, understanding these mechanisms and their evolutionary significance is essential for a fuller understanding of developmental plasticity and health of other animals including humans.

Acknowledgements

We thank Otso Huitu, Heiko Rödel, Janne Sundell and an anonymous referee for valuable comments on earlier drafts of this manuscript. Matti Häkkilä, Heidi Järvenpää, Tiina Nukarinen and Jarmo Ruutana are acknowledged for their contribution to field and laboratory work. Experimental Animal Unit of University of Jyväskylä and Konnevesi Research Station are acknowledged for providing research facilities. Centre of Excellence in Evolutionary Research and the Academy of Finland (grant 132190 to T.M.; 115961, 119200 and

103148 to E.K.) financially supported this study. The use of the animals adhered to ethical guidelines for animal research in Finland as well as all the institutional guidelines.

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Received 10 March 2011; accepted 21 November 2011
Handling Editor: Rolf Ims