Mechanisms for delayed density-dependent reproductive traits in field voles, *Microtus agrestis*: the importance of inherited environmental effects

Torbjørn Ergon, James L. MacKinnon, Nils Chr. Stenseth, Rudy Boonstra and Xavier Lambin

Reproductive traits of voles vary with the phases of the population density fluctuations. We sought to determine whether the source of this variation resides in the individuals or in their environment. Overwintering field voles (*Microtus agrestis*) from two cyclic out-of-phase populations (increase and peak phases) were sampled in early spring and bred in the laboratory for two generations under standardised conditions with ambient light and temperature. Monitoring of the source populations by capture-mark-recapture showed large differences in reproductive performance. In the increase area, reproduction started six weeks earlier, the probability of maturation of young-of-the-year was more than ten times higher during mid-summer, and reproduction continued nearly two months later in the autumn than in the peak area. These differences were not found to be associated with a difference in age structure of overwintered animals between the two areas (assessed by the distribution of eye lens masses from autopsy samples). Although the population differences in reproductive traits were to some degree also present among the overwintered animals in the laboratory, we found no difference in reproductive traits in the laboratory-born generations. There was a strongly declining seasonal trend in probability of sexual maturation both in the field and in the laboratory under ambient light conditions. However, in the field there were large population differences in the steepness of the seasonal decline that were not seen under the standardised laboratory conditions. We conclude that seasonal decline in maturation rates is governed by change in photoperiod, but that the population level variation in the shape of the decline is caused by a direct response to the environment and not due to variation in any intrinsic state of the individuals reflecting the environment experienced by the previous generation(s).

Since the first scientific description of small rodent population cycles by Elton (1924), much variation has been documented in the density fluctuations of different populations. Through time-series analysis, this variation has been described in terms of differences in the strength of direct and delayed density dependence on population growth rate (Bjørnstad et al. 1995, Turchin 1995, Stenseth et al. 1996, Stenseth 1999). However, less is known about the demographic mechanisms of the regulation. Indeed, the ecological mechanisms of the large variation in life histories of individuals within many animal populations are poorly understood (McNamara and Houston 1996).

In fluctuating small rodent populations, there is profound between-year variation in body size, timing of maturation and reproductive performance of individu-
als. In years with increasing population densities, overwintering animals generally start to breed earlier in the spring and more animals mature in their year of birth than in other years (Krebs and Myers 1974, Hansson and Henttonen 1985, Gilbert and Krebs 1991, Boonstra 1994). This general life-history pattern seems to be a universal characteristic of microtine population fluctuations and is also seen in some non-cyclic but multi-annually fluctuating populations (Agrell et al. 1992).

If the properties of individuals vary in relation to previous densities, there must be a ‘memory’ within the system. This memory may reside in the environment (interactions with predators, parasites or food resources) and/or within the population itself. The population may ‘remember’ past conditions in two ways. First, environmental conditions changing over time may affect demographic processes that alter the age structure of the population, and this may in turn affect future demographic rates. Boonstra (1994) and Tkadlec and Zejda (1998) found that the demographic processes commonly observed in fluctuating small rodent populations cause a shift in age structure towards older animals after peak population densities, and they argued that declines become inevitable because of senescence (i.e., the ‘senescence hypothesis’). Second, environmental conditions may cause a variation in the internal states of the individuals, through genetic selection and/or through persistent changes in the individuals’ physiological state. Assumptions of hypotheses for population regulation involving fluctuating genetic selection that generates delayed density dependence in reproduction, like Chitty’s (1960, 1967) polymorphic genetic-behavioural hypothesis and its variants (Krebs 1978), have not been supported empirically, and intraspecific variation in life-history traits of small rodents does not seem to have an important genetic basis (Boonstra and Boag 1987, Boonstra and Hochachka 1997). However, hypotheses involving time lags maintained by phenotypic changes and maternal effects have been little explored until recently. The environment in early life, including maternal effects operating during gestation and lactation, is often an important determinant of life histories in mammals (Bernardo 1996, Rossiter 1996, Inchausti and Ginzburg 1998), including microtines (Boonstra and Boag 1987, Boonstra and Hochachka 1997, Hansen and Boonstra 2000).

In this paper we examine the proximate causes of variation in reproductive performance of overwintering animals and their offspring in cyclic populations of field voles (Microtus agrestis). We tested whether variation in reproductive traits seen in the field can be explained by mechanisms whereby the memory of past conditions resides in the individual voles. Overwintering voles from two areas that differed in previous densities were bred under standardised conditions in the laboratory alongside monitoring the source populations in the field. A similar experiment was undertaken by Mihok and Boonstra (1992) on a fluctuating population of M. pennsylvanicus. However, whereas Mihok and Boonstra (1992) sampled voles from two different years (decline and increase) in the same area, we sampled voles simultaneously from two cyclic out-of-phase populations. First, we tested if variation in breeding performance seen in the field was maintained under standardised laboratory conditions. Secondly, we bred the voles for two generations to test whether the population differences would be reinforced by genetic and/or maternal effects. By comparing the age distributions through the distribution of eye lens masses in autopsy samples (cf. Hagen et al. 1980), we sought to assess the potential for senescence in causing the population differences.

Material and methods

Study system

Field voles (Microtus agrestis) were sampled from the Kielder and Kershope forests on the border between England and Scotland (55°13’. N. 2°33’. W). These man-made conifer forests have been planted over the last 70 yr and now have a mosaic of clear-cuts that are separated by dense spruce forest. Voles only inhabit the grassland clear-cuts, which are connected by road verges and fire breaks throughout the forest. The mesotrophic vegetation in these areas is dominated by Deschampsia caespitosa, Holcus lanatus, Agrostis spp. and Juncus effusus.

The two forests are in adjacent water sheds approximately 20 km apart within the larger (620 km²) forested area. Long-term monitoring of field voles in Kielder has shown cyclic population fluctuations with a 3–4-yr period (Petty 1992, Lambin et al. 2000). Although populations on nearby clear-cuts fluctuate in synchrony, out-of-phase populations are found within the larger forest region. Kershope forest has been one year ahead of Kielder since 1993 (Petty and Fawkes 1997, Lambin et al. 1998). The voles at Kershope reached high population densities in the autumn of 1996 and maintained high densities in 1997, while the population densities in Kielder were lower in 1996 but increased in 1997 (Fig. 1).

Sampling of parental animals for the laboratory

Voles for breeding in the laboratory and for autopsy were sampled from nine clear-cuts within a 50-km² area in Kielder forest (‘increase area’) and three clear-cuts 1–2 km apart in Kershope forest (‘peak area’) in two different periods, 12–24 March and 13–15 April 1997. Voles were caught in Ugglan Special multiple capture traps placed in active runways. Trapping continued on
the sites for 2–3 d to avoid sampling only the most trappable animals. On capture, animals were individually marked with ear-tags, weighed and their reproductive status was noted. Pregnancy of captured females was determined by swollen abdomen, parturition in the laboratory, or by autopsy.

A random subset of the March sample was autopsied. For determination of relative age (see Hagen et al. 1980), eyes from autopsied voles (freshly sacrificed) were fixed in 4% formaldehyde for one week and then stored in 70% ethanol until being dissected and dried for one week at 70°C. Eye-lenses were allowed to cool to room temperature in a desiccator where they were kept until being weighed as pairs on a Mettler™ M3 balance (precision 0.1 mg).

Laboratory procedures

While field sampling was taking place, animals were kept in single-sex, multi-animal cages (up to 10 individuals per cage) after capture until being transported to rearing facilities in Aberdeen, Scotland. In the laboratory, animals were caged in pairs in cages with sawdust and placed in sheds with windows and no heating.

Hence, photoperiod and temperature varied with the ambient conditions. Placement of the cages within the laboratory was randomised.

Animals were fed on fortified cereal pellets (‘Rat and Mouse No. 1’ from Special Diets Services; 14.7% protein) and provided with hay for bedding and extra food. Parental animals were also given apples and carrots for the first two weeks until they learned to drink from the water bottles. Each cage was supplied with a wooden nest box and two water bottles. The voles regularly chewed on the nest boxes, hence reduced teeth wear was not a problem. After one of the authors (TE) became infected with leptospirosis (Leptospira saxkoebing), all animals in the colony were treated with antibiotics (Tetramycine) in the first week of July.

Adults were weighed and checked for reproductive status every two weeks. Cages with pregnant females were checked every two to three days in order to determine the exact date of parturition. Females were classified as breeders if they gave birth in the laboratory or were shown to be pregnant by autopsy. The males were usually left in the cages with their mate during pregnancy and after parturition, but when there was shortage of sires, males were sometimes taken away to be paired with another female.

Juveniles were separated from their mother at 18–20 d of age, and one female offspring from each litter was selected at random for breeding in the next generation. These females were paired with non-paternal, known breeder, adult males from the parental generations. In some cases, when excess males were available, two females from the same litter were paired but only one of them (drawn at random) entered the analysis if both survived to parturition.

Population monitoring in the field

Eight sites in the increase-phase area and two sites in the peak-phase area were monitored before and after sampling to provide estimates of density trajectories and data on breeding and maturation in the field (the eight increase sites were part of another study, MacKinnon 1998). At each site, a 0.3-ha live-trapping grid was established, consisting of 100 Ugglan Special Mousetraps in a 10 × 10 configuration with 5-m spacing. The trapping regime followed the ‘robust design’ (Pollock 1982) with primary sessions at 27–31-d intervals consisting of six secondary sessions over 2 d. The first trapping session was at the end of March in the increase area and in mid-April in the peak area. Traps were pre-baited for 3 d, set between 06:00 and 08:00 and checked three times per day at 4-h intervals. The traps were not set overnight. Individuals were marked with a pair of uniquely numbered Hauptner™ ear-tags. The first time an animal was caught in each primary session it was weighed and checked for reproductive state.
Estimating timing of reproduction and maturation rates in the field data

In order to compare the pattern of maturation in the laboratory with maturation rates in the field, we employed multi-state capture-mark-recapture models (Hestbeck et al. 1991, Brownie et al. 1993) in the program MARK (White and Burnham 1999, White 2001). Males were classified as reproductive if they had scrotal testes. Females were classified as reproductive if they were lactating, if their pubic symphysis or vaginal opening indicated that they had recently given birth, or if their nipples indicated that they had recently weaned young. We primarily wanted to estimate maturation rate, and not the rate at which reproducing animals cease reproducing for the season. Hence, animals were classified as reproductive if they had been captured in reproductive condition in an earlier session, and the parameter in the likelihood function representing the transition probability from reproducing to non-reproducing state was fixed to zero.

Data from each trapping site were fitted with a separate model, and the most parsimonious models were selected based on Akaike’s Information Criterion adjusted for sample size, AICc (see Burnham and Anderson 1998). The seasonal variation in maturation probability of young-of-the-year females was modelled as a logit-function of capture date, assuming a monotonic decline in maturation probability over the season. This assumption was assessed graphically, and a complete time specific structure never increased the parsimony (i.e., never lowered the AICc) of the selected models. To simplify the model selection procedure, we started with a model structure for apparent survival (\( \phi \)) and recapture probability (\( p \)) that was found to be most parsimonious in standard Cormack-Jolly-Seber models without multiple states. A general model for maturation probability (different slope and intercept for the two sexes) was then used to determine whether additive or interacting effects of reproductive state on \( \phi \) and \( p \) would increase the parsimony of the models. With the new model for \( \phi \) and \( p \), we then searched for the most parsimonious model for the maturation probability. Alternative models for \( \phi \) and \( p \) were then again tested to ensure that the most parsimonious model had been found.

Since almost all overwintered animals matured during the first one-month interval between trapping sessions (April–May), we could not estimate the timing of onset of spring reproduction from the capture-mark-recapture data. To compare the onset of spring reproduction between the areas we relied on data on the frequency distributions of reproductive states of captured females. We also compared the seasonal patterns of reproduction by studying the recruitment of juveniles to the trapping sites.

Statistical analysis

As animals taken to the laboratory were sampled from a small number of sites within only one year and geographical region, we stress that the sampled individuals do not represent a random sample from particular ‘phases’ of the cycles as such, but rather from two areas having experienced contrasting densities in the previous year. Phase dependence is a likely cause of the population (area) differences, but we cannot make statistical inferences about the area differences. Our intention is to test whether population differences in the field were maintained under standardised conditions in the laboratory. Hence, in the analysis of the laboratory data we considered all sampled animals within each of the populations and sample periods as independent, and compare the population differences in the field with the population differences in the laboratory at the level of the individuals (not sampling sites).

Each response variable was investigated by two different models. First, to assess differences in population means, models with only population and sampling time were used. Second, models including intrinsic state variables (body mass and reproductive state, i.e., pubic symphysis, perforate/non-perforate vagina and pregnancy status) were used to search for underlying mechanisms behind the differences.

There was high mortality among the parental generation in the laboratory. A total of 69.5% \((n = 141)\) ‘increase-area females’ and 41.6% \((n = 113)\) ‘peak-area females’ died before they were paired or within 12 d after pairing. As the animals were transported and kept in multi-animal cages prior to pairing, and a possible disease may have been transmitted between individuals, these differences in mortality in the laboratory may have little relevance to the wild populations because individuals were not treated independently. However, females of the two populations were very different in their initial states, so care must be taken to avoid erroneous inferences due to experiment-induced bias caused by selective mortality. In order to reduce possible biases when testing for population differences in the laboratory, each observation was weighted with the inverse of the estimated probability of survival in the estimation (see Littell et al. 1996). Hence, the sum of weightings of animals of a particular characteristic divided by the total sum of weightings for all animals will be the same among the survivors as in the original sample (where all have equal weightings). Since the groups differed greatly in intrinsic state variables, survival probabilities used to calculate weightings were estimated with separate logistic models for each population and sample period, and the best models were selected by the lowest AIC. These models are given in Table 1.

As we did not have any prior knowledge of the importance of maternal effects, we considered models...
Table 1. Survival models (logit[probability of survival], binomial error distribution) used to calculate the weightings used to test for differences between samples of females. 95% confidence intervals of the parameters are given in brackets. Models were selected according to the lowest AIC. Weightings for each observation were calculated as the inverse of the predicted survival probability (see Material and methods), and the ratios between maximum and minimum weightings in each group are given in the right-hand column.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intercept</th>
<th>Pregnant = ‘no’</th>
<th>Capture body mass (g)</th>
<th>% survived of total</th>
<th>Ratio max/min weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak area – March</td>
<td>0.41 [-0.02, 0.85]</td>
<td>-1.76</td>
<td>-0.08 [-0.22, 0.03]</td>
<td>60% of 85</td>
<td>1.0</td>
</tr>
<tr>
<td>Increase area – March</td>
<td>2.36 [-1.63, 6.74]</td>
<td>-3.42, -0.30</td>
<td></td>
<td>24% of 91</td>
<td>3.7</td>
</tr>
<tr>
<td>Peak area – April</td>
<td>0.14 [-0.60,0.90]</td>
<td>+0.21 [0.08, 0.39]</td>
<td></td>
<td>53% of 28</td>
<td>1.0</td>
</tr>
<tr>
<td>Increase area – April</td>
<td>-6.30 [-11.32, -2.62]</td>
<td></td>
<td></td>
<td>42% of 50</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Results

Reproductive traits and demography in the field

The voles at Kershope reached high population densities in the autumn of 1996 and maintained high densities in 1997, while the population densities in Kielder were lower in 1996 but increased in 1997 (Fig. 1). To increase readability we will in the following refer to Kershope as the ‘peak area’ and Kielder as the ‘increase area’. The first spring born juveniles appeared more than six weeks earlier in the increase area than in the peak area (Fig. 2). The large difference in the onset of reproduction is also evident from the distribution of body mass and reproductive state of the overwintered animals taken to the laboratory in both March and April (see below). Young-of-the-year in the increase area continued to mature later in the season (Fig. 3), and reproduction continued for nearly two months later in the autumn than in the peak area (Fig. 2).
Initial characteristics of the laboratory sample

Distributions of eye lens mass (an index of age) from the autopsy (Fig. 4) were not significantly different between the two populations (two-sample Kolmogorov-Smirnov tests, *p* > 0.4). However, increase-area animals of both sexes had generally larger body mass (Fig. 5) and many females had already started to reproduce in the increase-area sample (Table 2).

Breeding performance of the overwintered generation

Frequency and timing of breeding in the lab

In the laboratory as in the field, the overwintered animals from the two populations showed different patterns of reproduction. A higher proportion of the increase-area females conceived and the increase-area females had shorter average time between pairing and parturition (26 vs 32 d) than the peak-area females (Fig. 6a; weighted Cox proportional hazard regression, population effect: *Z* = 2.44, *p* = 0.015). Excluding nine females that died within 12 d after pairing, 95% of the increase-area females vs only 77% of the peak-phase females either gave birth or showed signs of pregnancy when autopsied (weighted logistic model, population effect: *χ*^2^ = 6.40, 1 df, *p* = 0.013). Non-breeding females lived up to 205 d (1st to 3rd quartile: 32 to 111) together with their mate. Breeding probability of peak-area females increased significantly with body mass at capture (logistic regression: *χ*^2^ = 5.15, 1 df, *p* = 0.023).

Only the largest peak-area females had an estimated breeding probability equal to the increase-area females (Fig. 7).

Increase-area females were still heavier after parturition than peak-area females (weighted linear model, population effect: *F* = 9.05; 1, 82 df; *p* = 0.004; 95% c.i., increase area: 38.3 ± 1.2 g, peak area: 35.5 ± 1.4 g). Thirty of the breeding peak-area females and 27 of the increase-area females had a mate at parturition of the first litter, and hence the opportunity to re-conceive. All of these females became pregnant except for four increase-area females that only had a mate for 4–10 d after giving birth to their first litter. Time between first and second parturition ranged from 19–42 d (mean = 21.2 d) but there was no significant pattern in this variation.

Litter size

Increase-area females had on average larger litters (least square mean = 4.5; 95% c.i. [4.2, 4.9]) than did peak-area females (least square mean = 4.0; 95% c.i. [3.6, 4.4]; weighted log-linear model, population effect: *χ*^2^ = 4.04, 1 df, *p* = 0.044; sampling month: *χ*^2^ = 2.83, 1 df, *p* = 0.093). Animals with closed pubic symphyses when captured had smaller litters (mean = 3.9; 95% c.i. [3.6, 4.2]) than those with open pubic symphyses when captured (mean = 5.2; 95% c.i. [4.6, 6.0]; log-linear model: *χ*^2^ = 14.4, 1 df, *p* = 0.0002). When state of pubic symphysis was included in the model, the population effect was no longer significant (*χ*^2^ = 0.99, 1 df, *p* = 0.32; interaction effect: *χ*^2^ = 2.75, 1 df, *p* = 0.10). The size of litters conceived in the laboratory was also related to...
pregnancy status at capture in the increase-area sample (there were very few pregnant females in the peak-area sample, see Table 2); mean of 5.2 (95% c.i. [4.4, 6.1]) among those pregnant at capture versus 4.1 (95% c.i. [3.6, 4.7]) for those not pregnant at capture. Litter size of those females that re-conceived in the laboratory increased significantly from first to second parturition (average increase: 1.1 pups; Wilcoxon matched pairs test: \( Z = 4.30, p < 0.0001 \)). Together, these results suggest that the population difference in litter size may be due to a parity effect, as a larger proportion of the increase-area females had previously bred in the field.

Breeding performance of the laboratory-born generations

As there was large variation in date of birth of the laboratory-born females, and since date of birth differed between the two populations, date of birth was used as a covariate in all further analysis.

Body mass at weaning

Body mass at weaning (18–22 d old, age included as a covariate in the model) of the first laboratory-born generation (F1s) generally decreased with increasing number of littermates (95% c.i. \( -0.73 \pm 0.69 \) g per additional littermate) and increased with date of birth (95% c.i. \( 0.09 \pm 0.05 \) g d\(^{-1}\)). However, there was no difference between the two populations (model including littermates and date of birth, population effect: \( F = 1.16; 1, 82 \) df; \( p = 0.28 \), alone: \( F = 1.68; 1, 84 \) df; \( p = 0.20 \)).

In the second lab-born generation (F2s), body mass at weaning decreased with increasing date of birth (95% c.i. \( -0.16 \pm 0.08 \) g d\(^{-1}\)), and estimated weaning mass was 2.47 g (± 2.12 g; 95% c.i.) higher for the peak-area females than for the increase-area females born on the same date.

Frequency and timing of breeding – F1s

Four of the 49 breeding peak-area parental females did not wean any daughters, and a further two of the peak-area F1-females died shortly after pairing. Of the remaining F1 females, 49% (\( n = 41 \)) of the increase-area females and 55% (\( n = 43 \)) of the peak-area females conceived (revealed by parturition or autopsy) in the laboratory (Fisher’s exact: \( p = 0.7 \)). There was no significant population effect on breeding pattern (Fig. 6b, Cox proportional hazard regression, population effect: \( Z = 0.12, p = 0.9 \)). However, date of birth under the ambient photoperiod conditions had a highly significant effect on breeding probability (logistic regression model: \( \chi^2 = 16.05, 1 \) df, \( p < 0.0001 \)). Females born early in the season had a much higher estimated probability of becoming a breeder than those born late in the season (Fig. 8a).

Frequency and timing of breeding – F2s

In the F2 generation, four of the 21 (19%) increase-area females versus seven of the 24 (29%) peak-area females conceived (Fisher’s exact test: \( p = 0.5 \)). As in the F1 generation, there was a decreasing probability of breeding for F2 females born later in the season (Fig. 8b; logistic regression model: \( \chi^2 = 4.77, 1 \) df, \( p = 0.029 \)), but there was no significant population effect on the breeding pattern (Fig. 6c; Cox proportional hazard regression, population effect: \( Z = 1.03, p = 0.3 \)). However, a different pattern in timing of breeding was generally seen in the F2 generation than in the F1 generation. Only one F2 female raised pups before mid-August, even though they were paired 50–95 d earlier. This female had only one pup in mid-July, whereas all other females had three pups. The five
earliest born F2 females had their first litter killed by one of the parents, but all except one of these pairs raised young later in the season. Infanticide of own litters occurred only twice among the 90 breeding parental females and never among the 44 breeding F1 females.

Non-breeding females remained small throughout the summer, and by 1 August they were significantly smaller (mean = 22.8 g, SD = 3.1) than breeding males (mean = 28.0, SD = 2.4, one pregnant female omitted; two-tailed t-test: p < 0.0001).

**Litter size in the laboratory generations**

There was no significant difference in average litter size of F1s between the two populations (increase area 3.9 vs 4.1 for peak area; $\chi^2 = 0.15$, 1 df, $p = 0.7$). All females in the F2 generation had three offspring except one increase-area female having only one pup.

**Cross-generational correlations**

Despite large variation in reproductive traits within both the parental generation and the first laboratory-born generation, there was no indication of any strong effects carrying over between the generations (Table 3). The weak correlation between time before parturition in the parental generation and the pre-weaning growth rate of their daughters (Table 3) may be a date (photoperiod) effect. Heavier animals at capture also tended to be heavier after giving birth, and laboratory-born animals with higher pre-weaning growth rate also tended to be heavier after parturition.

**Discussion**

We have demonstrated large variation in the seasonal trends in reproductive traits between female field voles from two nearby cyclic populations that had experienced contrasting densities in the previous year. However, except for the parental laboratory generation, reproductive traits varied only seasonally under stan-
standard laboratory conditions. Hence, the large population differences in maturation rates that we found in the field are unlikely to have been caused by maternal or genetic effects, as assumed by the maternal effects hypothesis for population cycles (Boonstra and Boag 1987, Boonstra and Hochachka 1997) and Chitty’s fluctuating selection hypothesis (Chitty 1960, 1967).

Although we cannot make statistical inferences about the phase of the cycles from this study, the reproductive traits that we have been investigating (onset of spring reproduction and maturation of young-of-the-year) are known from many studies to vary consistently with the phase of small rodent cycles (see review in Krebs and Myers 1974, Hansson and Henttonen 1985, Bernshtein et al. 1989, Gilbert and Krebs 1991, Boonstra 1994).

Our field observations are in agreement with these general findings; later onset of spring reproduction and ‘poorer’ reproduction when the populations have gone through high densities.

**Overwintered animals – does the memory for delayed density dependence reside in the individual voles?**

Onset of spring reproduction in the field was more than a month later in the peak area than in the increase area. The large differences between the sites are shown by body mass distributions, frequency of reproductive states among overwintered animals when sampled and the time that the first juveniles in the spring appeared. Such differences in onset of reproduction were also found under the standardised laboratory conditions. Increase-area animals had also a larger proportion of breeders as well as larger litters in the laboratory. Our evidence indicates that the latter may be due to the fact that more animals in the increase area had previously reproduced in their lives.

Although the population differences in onset of reproduction were much smaller in the laboratory than in the field, the clear differences between the populations seen in the laboratory must represent different states of the animals when they were sampled. These initial state differences may have resulted in larger differences in reproductive performance in a natural but shared environment (or with a different experimental protocol). However, it is not clear from our experiment at what stage the individuals’ internal state diverged between the populations. On the one hand, the state differences may have been shaped early in the life of the individuals or resulted from genetic differences, in which case there would be a time-lag residing in the individuals. Hansson (1989) reported that laboratory-born *Microtus agrestis* started to breed two months earlier in the spring and had a higher frequency of winter breeding under laboratory conditions with ambient light than wild-born females living under the same conditions. This suggests that the environment in early life may be an important determinant of onset of spring reproduction. On the other hand, since some of the voles had already started to reproduce in the increase area when they were sampled in the field, it may also be that the increase-area animals had reacted to some cue in the environment just before sampling, and that the peak-area animals had not yet received this stimulus. The fact that the smallest peak-area animals failed to breed in the laboratory supports the latter suggestion – that the smallest animals may not yet have received an environmental stimulus to initiate spring growth and reproduction.

Boonstra (1994) and Tkadlec and Zejda (1998) found that low juvenile recruitment during the peak phase of
Table 3. Spearman rank correlations between reproductive traits of females within and across the parental (mothers) and the first lab-born (daughters) generation. The top number is the correlation coefficient for both populations pooled, the middle is for the peak-area population, and the lower number is for the increase-area population. Asterisks indicate significance levels; *: \( p < 0.05 \), **: \( p < 0.01 \), ***: \( p < 0.001 \).

<table>
<thead>
<tr>
<th>Pooled peak increase</th>
<th>Parental generation</th>
<th>F1 generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time before parturition</td>
<td>Litter size</td>
</tr>
<tr>
<td>Parental generation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litter size</td>
<td>−0.42***</td>
<td>−0.38**</td>
</tr>
<tr>
<td>Mother body mass at capture</td>
<td>−0.19</td>
<td>0.21</td>
</tr>
<tr>
<td>Mother body mass after parturition</td>
<td>0.11</td>
<td>0.15</td>
</tr>
<tr>
<td>F1 generation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-weaning growth rate</td>
<td>0.25*</td>
<td>−0.35**</td>
</tr>
<tr>
<td>Age at first parturition</td>
<td>0.13</td>
<td>−0.30</td>
</tr>
<tr>
<td>Litter size</td>
<td>0.12</td>
<td>−0.08</td>
</tr>
<tr>
<td>Mother body mass after parturition</td>
<td>0.20</td>
<td>−0.02</td>
</tr>
<tr>
<td></td>
<td>−0.29</td>
<td>−0.15</td>
</tr>
<tr>
<td></td>
<td>−0.24</td>
<td>−0.16</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>−0.01</td>
</tr>
<tr>
<td></td>
<td>−0.13</td>
<td>0.53*</td>
</tr>
</tbody>
</table>

Laboratory-born animals – there is no support for maternal or genetic effects

Although the parental generation showed clear differences in reproductive performance in the laboratory, these differences did not carry over to the laboratory-born generations. In fact, despite large variation in reproductive traits in all generations, there were no convincingly strong cross-generational correlations for any trait. Thus, maternal and genetic effects are not major sources of variation in reproductive traits of these cohorts under the laboratory conditions. Hence, the notion that maternal effects are important determinants of demographic traits in small rodent populations in general (Boonstra and Boag 1987, Boonstra and Hochachka 1997) is not supported by our study. Boonstra and Hochachka (1997) found strong maternal influence on growth rate and age at maturity of collared lemmings (*Dicrostonyx groenlandicus*) under laboratory conditions. However, such effects remain to be demonstrated under natural conditions.

The most prominent patterns seen in the laboratory-born generations were the differences between the generations and the changes over the season. In both of the laboratory-born generations, the estimated probability of breeding declined with date of birth of the female. All F2 females that raised any pups (except for one female having a single pup) did not do so until end of August to end of September. The only environmental change over time in the laboratory was that of seasonal change in ambient light conditions and temperature. Change in photoperiod is known to be an important cue for reproductive regression and inhibition of maturation before the winter in several species of voles (reviewed in Bronson and Heideman 1994) including field voles (Spears and Clarke 1988). However, regulation of reproduction by natural change in photoperiod during mid-summer has, to our knowledge, not been previously described.

The seasonal decline in maturation seen in the laboratory closely resembles the pattern seen in our live-trapping sites, especially in the peak area where maturation ended early in the season. Spears and Clarke (1988) suggested, based on their findings of
strong heritability of photo-responsiveness on maturation of juvenile field voles, that phase-dependent variation in the seasonal reproductive pattern in cyclic vole populations may be due to genetic differences (see also Nelson 1987, Bronson and Heideman 1994). However, in our study, the large season-specific difference in maturation between the areas was not maintained under the standardised laboratory conditions. This suggests that phase-dependent variation in juvenile maturation is not genetically based but rather caused by differences in the environment.

Mihok and Boonstra (1992) reported that female meadow voles (Microtus pennsylvaniaicus) from a low year after a severe winter decline had a much poorer breeding performance under laboratory conditions than females sampled during a strong increase the following year. In their study, however, low-year animals were sampled in early June and July (50/50 overwintered/young-of-the-year), whereas the increase-year animals were sampled at the start of the breeding season in May and had all overwintered. In the light of our own results, it is possible that the difference in reproductive performance observed by Mihok and Boonstra (1992) is due to seasonal effects rather than to phase-dependent differences.

**Possible environmental effects on reproductive traits in voles**

Although deterministic seasonal change in photoperiod can alone cause the seasonal trends in maturation probability, environmental mechanisms must be invoked to explain the large between-year/site variability in life histories of cohorts born in mid-summer cohorts which we found in this study.

Social constraints may affect juvenile maturation in the field (Krebs and Myers 1974, Morris 1989, Pusenius and Viitala 1993, Boonstra 1994). At the beginning of the year, there were differences in density between our two study areas, so the differences in maturation rate may partly have been due to direct density-dependent social suppression of maturation (e.g. Boonstra and Rodd 1983, Rodd and Boonstra 1988, Boonstra 1989, Gilbert and Krebs 1991). However, the difference in maturation frequencies was largest late in the year when the densities had more or less converged. Nor can social suppression of breeding explain why all overwintered animals in the peak area delayed maturation until late in the spring. The above points to the importance of extrinsic effects.

Suppressed reproduction and delayed maturation in microtines have been suggested to be an adaptive response to high risk of predation, and several laboratory studies have found strong effects of mustelid odours on reproduction (Ylönen 1989, Korpimäki et al. 1994, Koskela and Ylönen 1995, Mappes and Ylönen 1997). However, such an effect may be a laboratory artefact with little relevance for natural populations (Hansson 1995, Lamb et al. 1995, Korpimäki and Krebs 1996). A recent field experiment failed to find any effects of mustelid odours despite simulating very high predator density, which led the authors to conclude that their previous findings in the laboratory had little relevance (Mappes et al. 1998).

Feore et al. (1997) found that non-lethal cowpox infections in bank voles (Clethrionomys glareolus) and wood mice (Apodemus sylvaticus) increased the time before first litter under laboratory conditions. Such effects may be profound under natural conditions and may have interacting effects with, e.g., food availability. Cowpox is highly prevalent in our study system (Cavanagh et al. unpubl.).

Food quality and/or availability directly affect the physiological state of the individuals and may cause a delay in maturation (Berger et al. 1977, 1981, Gallacher and Schneeman 1986, Andreassen and Ims 1990, Ryan 1990). Negus et al. (1977) provide evidence that considerable variation in onset of reproduction in M. montanus between years may be related to the availability of green plants in the spring, which in turn is related to the time of snow melt. The phenolic plant compound, 6-methoxy-2-benzoxazolinone (6-MBOA), which is present in all growing grasses and sedges but has no nutritional value (Moffatt et al. 1991, Nelson 1991), is known to enhance gonadal development and trigger reproduction in several north-American Microtus species (Sanders et al. 1981, Korn and Taitt 1987, Gower and Berger 1990, Nelson and Blom 1993, Meek et al. 1995). Korn and Taitt (1987) found that Microtus townsendii in natural populations that were fed with oats coated with 6-MBOA started to breed four weeks earlier than voles in a control site 200 m away where oats coated with the solvent only were provided. Several mechanisms may cause a delayed response in food quality and/or availability to earlier grazing; e.g. quantitative reduction and slow recovery (Kalela and Koponen 1971, Oksanen and Oksanen 1981, Moen et al. 1993), depletion of the plants’ stored resources in the roots or altered allocation of resources to growth and defence (Herms and Mattson 1992), and induced plant resistance (Karban and Baldwin 1997). It is also well known from agricultural science that harvesting or grazing in a critical period in the autumn can interrupt cold acclimation, and thereby strongly reduce tiller survival and grass production in the following spring (Sheafler and Marten 1990, Sheafler et al. 1992, Shimada 1994). Bergeron and Jodin (1993) found a 52% reduction of green biomass in the spring after manipulating high densities of Microtus pennsylvaniaicus in enclosures the previous year.

To understand the mechanisms of population fluctuations of microtines, there is clearly a need for both better descriptions of the direct and delayed density-de-
pendent structure of variation in life-history traits, as well as studies on the individual level responses to the environment. Diseases and food quality/availability may impose severe constraints on the individuals' performance, and they therefore represent plausible agents to cause variation in reproductive traits. However, the role of diseases and food quality/availability in the population dynamics of microtines is as yet largely unexplored.

Acknowledgements – The laboratory part of this work was funded by a special grant from the Univ. of Oslo to NCS and by support from the Natural Sciences and Engineering Council to RB. The field monitoring was supported by a studentship (to JLM) and grants from Natural Environmental Research Council, UK to XL. The Centre for Advanced Study of the Norwegian Academy of Science and Letters together with the Univ. of Oslo supported RB to visit Oslo and the field sites in Scotland. TE has been supported by the Norwegian Science Foundation. Rearing facilities were generously provided at Culterry Field Station and we thank Robert Donaldson and Sue Way for assistance with maintaining the animals. We thank George Batzli, Karen E. Hodges, Robert Moss and Nigel Yoccoz for constructive comments on the manuscript.

References


196 OIKOS 95:2 (2001)


