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Does reproduction accelerate the growth of eye lens mass in female voles?

Eva Jánová [1], Dana Havelková [2] & Emil Tkadlec [3]

[1] **Masaryk University Brno, Faculty of Sciences, Department of Zoology and Ecology, Kotláø ská 2, 611 37 Brno, Czech Republic; Department of Mammal Ecology, Institute of Vertebrate Biology, AS CR, Kvtná 8, 603 65 Brno, Czech Republic**

[2] **Department of Population Biology, Institute of Vertebrate Biology, AS CR, 675 02 Studenec 122, Czech Republic**

[3] **Palacky University, Department of Ecology and Environmental Sciences, tø. Svobody 26, 771 46 Olomouc, Czech Republic**

Corresponding author : Janová Eva, e-mail : janova.eva@seznam.cz

ABSTRACT. Although the eye lens mass method has long been used for determining age in small rodents from natural populations, the effects of breeding on growth rates of lenses are rarely considered. Under laboratory conditions, we examined eye lens mass in two groups of 100- day old females of the common vole (*Microtus arvalis*) : one comprising the females that have already bred, each delivering two litters, and another comprising controls that did not reproduce. The breeding females were heavier than the nonbreeding ones and also had heavier dried eye lenses. However, the effect of breeding on eye lens mass disappeared when the variation in body mass was accounted for in the statistical model. The total number and mass of offspring that the females produced did not affect the lens mass. We conclude that different reproductive histories did affect the growth of eye lens in female common voles through its influence on body size. These results suggest that besides age, the construction of calibration curves for aging voles in natural populations should also include individual body mass as an additional covariate to account for variation in body mass due to differences in reproductive condition.

KEY WORDS : age estimation, eye lens mass, reproduction

INTRODUCTION

Demographic structuring based on age is an important concept in population ecology and biological conservation. Age determination by measuring the mass of eye lens is a method widely used in many mammals (Lord, 1959; Bothma et al., 1972; Broekhuizen & Maaskamp, 1979), including small microtinae rodents (Martinet, 1966; Lelouarn, 1971; Hagen et al., 1980, Adamczewska-Andrzejewska, 1981; Moravec, 1985; Gliwicz, 1994; 1996; Takahashi & Saitoh, 1997). This method is usually considered as one of the most reliable ones among those available for field research (Adamczewska-Andrzejewska, 1973; Gurnell & Knee, 1984). At the same time, it is well-recognised that it also has significant limitations in animals with varying biological rates, such as somatic growth rates and developmental rates (Meunier & Solari, 1972; Hansson, 1983a). In particular, short-lived rodents, such as voles, which exhibit large cohort variation in body growth and reproductive output over the year, are difficult to age even with this method. Large variation in biological rates, when translating into lens mass growth, may lead to large inaccuracies in estimated ages between individuals.

Typically, female voles from spring cohorts have the fastest somatic growth and sexual development and their life-time reproduction is concentrated very early in life. In contrast, autumn-born females grow slowly and enter reproduction after overwintering at an advanced age, concentrating life-time reproduction late in life (Bergstedt, 1965; Gliwicz et al., 1968; Martinet & Spitz, 1971; Myllymäki, 1977; Gliwicz, 1994; Tkadlec & Zejda, 1998a; b). Photoperiod and food quality are usually thought to be the major triggering factors driving the acceleration of biological rates in spring-born animals (Meunier & Solari, 1972; Martinet & Spitz, 1971; Hansson, 1983b; Batzli, 1986). Indeed, the growth of eye lenses has been observed to be faster in spring-born voles than later-born voles (Adamczewska-Andrzejewska, 1973; Meunier & Solari, 1972; Hlaváč, 1979; Moravec, 1985).

It remains an issue whether the acceleration of biological rates early in life may have negative consequences to processes late in life. A related concept of reproductive costs (Williams, 1966; Stearns, 1976; Koivula et al., 2003) implicitly assumes that reproduction, being definitely among the very demanding life periods coupled with high metabolic rates, contributes significantly to the acceleration of biological rates and is, in various ways, paid for later in life. If so, the breeding females should have heavier lenses and appear older than the nonbreeding females of the same age. However, since most females in spring populations are in reproductive condition, observations from natural populations do not allow discriminating between the effects of reproduction per se and other factors connected with the ongoing season.

The main objective of the present paper is to disentangle the effects of reproduction from the effects of other factors, such as daylight and food, on biological rates by measuring the growth of eye lens mass in breeding and nonbreeding captive female voles. The hypothesis that reproduction accelerates biological rates predicts that breeding females will have heavier lenses than nonbreeding ones of the same age.

MATERIALS AND METHODS

Experiment

The experimental animals originated from F1 and F2 generations produced by wild-caught common voles (*Microtus arvalis*, Pallas 1778) bred in captivity at 14 hours of light, 10 hours of dark, and a temperature of 20 ± 2°C. A special palletised food for voles, based on cereals, dry lucern, dry milk, vitamin and mineral supplement, was provided *ad libitum*. Immediately after weaning at 20 days of age, 54 females were paired with breeding males and kept as monogamous pairs until they gave birth to two litters. Two females died and the six others which did not reproduce were removed. By 100 days of age, reproduction occurred in 46 females, two of them reproduced only once, one female was in advanced pregnancy with a second litter and one other aborted the second litter. Delayed removal of a male caused third pregnancies in three females. The different number of litters and offspring produced were considered in calculating reproductive effort (RE). Another 59 females were kept in cages alone as nonbreeding controls, with 54 of them surviving to the required age. Surviving females from both samples were sacrificed at 100 days of age. Eyeballs were removed and then fixed in 10% formalin for more than 3 weeks. After draining out excessive fluid by filter paper, the preserved lenses were weighed on an analytical balance to the nearest 0.1mg (the formalin lens mass). The lenses were then dried at 55°C for 24 hours and weighed again (the dried lens mass).

Statistical analysis

The experimental and control females were produced by 36 mothers. Consequently, there were 36 sibling groups, within which the data were likely to be correlated, violating the basic assumption of data independence. Therefore, to avoid pseudo-replication when analysing the treatment differences in female body mass, formalin lens mass, and dried lens mass, we fitted generalized linear mixed models assuming normal error distribution and including mother's identity as a random effect, using procedure MIXED from the package SAS 9.1.3 (Sas Institute Inc., 2004; Littell et al., 1996). Both the intercept and treatment effect were assumed to have a random component. We used restricted maximum likelihood method to estimate variance components. According to theory, breeding females, by having a higher metabolic rate, were expected to have both higher body and lens masses. We therefore adopted one-tailed significance testing procedure by applying the F-test, with the Kenward-Roger's method for computing the denominator degrees of freedom. The reproductive effort of a female was calculated according to the Millar (1977) and slightly modified as $RE = N\ W_w{}^{0.75}\ (m^{0.75})^{-1}$, where N = total litter size, W_w = the mass of all offspring at weaning divided by N, and m = adult female mass at the time of sacrifice, mostly shortly before or after weaning the second litter. The W_w was computed as the quotient of the total mass of weaned offspring and total litter sizes, because the nestlings which did not survive to weaning should be considered as well.

RESULTS

As expected, the females that bred were, on average, heavier by about 20% than those that did not ($F_{1,26.2}$ = 10.86, p = 0.003, Table 1). Because there were no differences in body mass at the beginning of the experiment ($F_{1,10}$ = 0.30, p = 0.60), the higher body mass of breeding females was most likely due to reproduction. Females that were heavier had larger both formalin ($F_{1,89.7}$ = 4.97, p = 0.028, Fig. 1a) and dried lens masses ($F_{1,37.3}$ = 6.59, p = 0.014, Fig. 1b). While there was no difference in formalin lens mass between the breeding and nonbreeding females ($F_{1,91.6}$ = 0.20, p = 0.66), the difference in dried lens mass was significant ($F_{1,20.7}$ = 2.91, p = 0.10), suggesting that reproduction does have an effect on the growth rate of lenses, enhancing their mass by about 5%. The treatment effect on dried lens mass, however, disappeared completely in a combined statistical model including both treatment and the body mass (treatment : $F_{1,25.6}$ = 0.94, p = 0.34; body mass : $F_{1,68.2}$ = 5.94, p = 0.017). As the regression of eye lens mass on body mass could differ between the two groups, we tested for interaction between the treatment and body mass (test of heterogeneity of slopes). We found no difference in slopes for body mass of breeding and nonbreeding females (interaction treatment*body mass : $F_{1,64.7}$ = 0.35, p = 0.56). This indicates that reproduction influences the growth of lenses through its overall effect on body mass (Fig. 1). In other words, there is an allometric relationship between the dried eye lens mass and body size of voles. The estimated treatment effect for dried lenses in females at 100 days of age was 0.12mg which may correspond to a bias of about 50 days of age and progressively more at later ages because of the non-linearity in the age-lens regression. Neither formalin nor dried lens mass depended on the total mass of all offspring at weaning (formalin : $F_{1,42.5}$ = 0.35, p = 0.55; dried : $F_{1,24}$ = 1.30, p = 0.27) or the total number of offspring produced at weaning (formalin : $F_{1,42.8}$ = 0.12, p = 0.73; dried : $F_{1,26}$ = 1.21, p = 0.28). Female's body mass at 100 days of age tends to be negatively related to RE ($F_{1,35.8}$ = 3.96, p = 0.054); however, neither the formalin ($F_{1,42.7}$ = 0.05, p = 0.83) nor the dried lens mass ($F_{1,39.3}$ = 0.02, p = 0.90) depended on the total RE.

DISCUSSION

Exploring the differences in body mass and eye lens mass in breeding and nonbreeding female common voles, we found that the breeding females had heavier eye lenses but at the same time, that this effect could be explained fully by the increased body mass of breeding females compared to the nonbreeding ones. The resulting body size in breeding females at 100 days of age seems to be negatively affected by their reproductive effort, perhaps because of the high metabolic requirement during lactation. We conclude that breeding, no matter the size of reproductive allocation, can accelerate the eye lens mass growth rate of mothers and hence, the body mass of individuals at capture should be considered as an additional covariate in calibration equations for aging voles in natural populations.

TABLE 1

The comparison of least square means with 95% confidence intervals for body and lens masses between breeding and nonbreeding female common voles in captive experiments estimated by fitting generalized linear mixed models.

Variable	Breeding		Nonbreeding	
	Mean	95% c.i.	Mean	95% c.i.
Body mass at weaning (g)	14.4	13.4 – 15.3	14.0	13.2 – 14.9
Body mass at 100 days (g)	30.2	27.8 – 32.6	24.7	22.4 – 27.1
Formalin lens mass (mg)	10.43	10.10 – 10.76	10.34	10.1 – 10.68
Dried lens mass (mg)	5.62	5.38 – 5.85	5.35	5.12 – 5.59

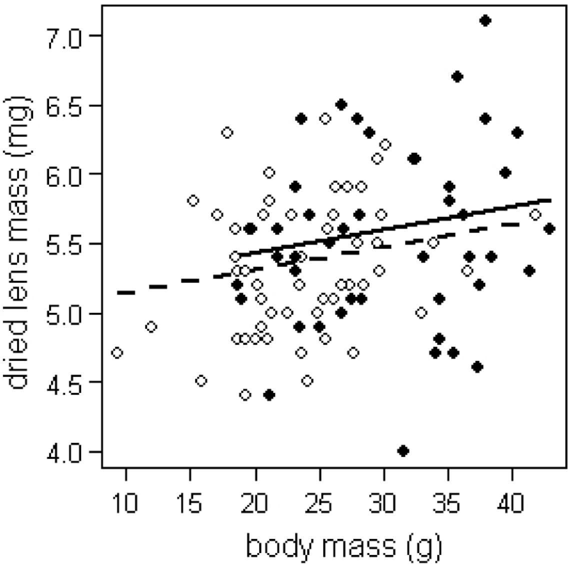

Fig. 1. – Relationship between the dried lens mass and body mass estimated by fitting generalized linear mixed model for breeding (solid line) and nonbreeding female common voles (dashed line) at 100 days of age. The circles are the observed data for breeding (closed circles) and nonbreeding females (open circles).

In voles, the sexual maturity of individuals and their entering into reproduction is connected with the increased body size (Zejda, 1971; 1992; Markowski, 1980). The rapid recruitment of early maturing individuals into the population and their fast body growth is usual in the spring (Gliwicz et al., 1968; Martinet & Spitz, 1971; Myllymäki, 1977; Gliwicz, 1994; Tkadlec & Zejda, 1998a; b). An accelerated growth of the eye lenses occurs in the same part of the year (Meunier & Solari, 1972; Hlaváč, 1979; Moravec, 1985) and results in higher lens mass in mature as compared to immature animals (Hanson, 1983b). We found that these two processes operated together in laboratory-bred animals as well, even though only the dried lens mass turned out to be sensitive enough to respond to breeding. This emphasizes again that aging of voles should indeed be based on the dried lens mass rather than the wet (formalin) one (e.g., Mallory et al., 1981, Jánová et al., 2003) if the higher precision of estimates is required. The differences in eye lens mass between breeding and nonbreeding are likely to increase progressively with age and individuals born towards the end of breeding season. In late summer and autumn, body growth rates begin to slow down in preparation for overwintering and the age of sexual maturation is delayed until the next year. Age at first reproduction and sexual maturation is also under a strong influence of population density which fluctuates greatly in voles (Mallory et al., 1981; Boonstra, 1989; Tkadlec & Zejda, 1995; Tkadlec & Zejda, 1998a).

Body mass tended to be negatively related to RE, most likely because of higher energetic demands on a female's body when weaning a larger litter (McInroy et al., 2000). However, the effect of variation in RE on body mass appeared to be rather weak, with no effect on the eye lens mass at all, even though body and lens masses were observed to be correlated. Perhaps, the variation in RE imposed by the experimental design was too small in order to observe any response beyond that of the body size.

The eye lens method of aging is widely used in small mammals. We demonstrated that the different reproductive histories among female voles do impose a bias in estimates of age through their effect on body size. Because of the allometric relationship between the mass of eye lenses and body size, larger voles, usually those that breed, appear to be older than smaller, usually nonreproducing voles. Hence, reproduction does accelerate the growth of eye lens mass in female voles. Assuming that the similar allometric relationship exists for males as well, which is very likely, we suggest that the construction of calibration curves for aging voles in natural populations should also include individual body mass as an additional covariate to account for variation in body mass due to differences in reproductive condition.

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