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# Age determination in the Smith's red-backed vole, *Eothenomys smithii*, using optic lens weight

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**Abstract**. A technique for age determination based on the dry weight of the optic lens was tested in Smith's red-backed vole, *Eothenomys smithii*. The model equation $W=a+b\log_{10}A$ ($W$: lens weight in mg, $A$: age in days, $a$ and $b$: parameters to be estimated from data) was applied to our data from 65 known-age laboratory-reared voles. As a result, the predicted age in days ($\hat{A}$) at a given lens weight ($W$) could be calculated from the equation $\hat{A}=10^{(W+1.415)/2.131}$. For example, an individual with a lens weighing 3.22 mg was estimated as having a predicted age of 150 days, and the 95% confidence interval at 150 days was calculated to be 17 days (142-159 days, or 11.3% of the predicted age) for the mean prediction and 144 days (94-238 days, or 96.0% of the predicted age) for the individual prediction. Lens weight can, it appears, provide the best age criterion at present, particularly in rodents with rootless molars such as *E. smithii*.

**Key words**: age determination, *Eothenomys smithii*, lens weight, Microtinae, red-backed vole.

Information concerning age is a very important aspect of ecological studies of wild animals. Many methods for age estimation have been proposed and used in various mammalian species (see Morris 1972 for review). Among the various methods available, the lens weight technique has been evaluated as a useful and powerful technique in small to medium-sized mammals, such as rodents (Hagen *et al*. 1980 for *Microtus oeconomus*, Okamoto 1980 for *Rattus norvegicus*, Tanikawa 1993 for *Rattus rattus*, Takada 1982a for *Mus musculus molossinus*, Takada 1982b for *Apodemus speciosus*), and lagomorphs (Load 1959 for *Sylvilagus floridanus*, Dudzinski and Mykytowycz 1961 for *Oryctolagus cuniculus*, Connolly *et al*. 1969 for *Lepus californicus*, Bothma *et al*. 1972 for *S. floridanus*, Hearn and Mercer 1988 for *Lepus arcticus*, Ando *et al*. 1992 for *Lepus brachyurus*).

Smith's red-backed vole, *Eothenomys smithii*, of the subfamily Microtinae, is endemic to Japan, where it occurs widely in forested areas of Kyushu, Shikoku, and western and central Honshu (Kaneko 1992). In contrast to the Japanese gray red-backed vole, *Clethrionomys rufocanus bedfordiae* (Abe 1976) and the large Japanese field mouse *A. speciosus* (Hikida and Murakami 1980), neither the molar root ratio, nor the molar wear pattern, can be employed as an age criterion in *E. smithii*, since its molars are rootless and grow persistently. No information has been available on age estimation in *E. smithii*. For the experiment described here, age was estimated for individual *E. smithii*, based on the optic lens weight of known-age individuals, using statistical treatments recommended by Dapson (1980).

## Materials and Methods

### 1. Lenses

The *Eothenomys smithii* used for this study were obtained from a laboratory colony which originated from wild voles live-trapped on Mt. Wakasugi in Fukuoka Prefecture, northern Kyushu. The colony was maintained under controlled conditions, *i.e.*, temperatures of 15–20 °C and photoperiods of 12–13 hr light : 12–11 hr dark (Ando *et al.* 1988). A total of 65 voles (33 males, 32 females) ranging in age from 20 to 600 days were killed with ethyl ether. Both right and left eyes were dissected out and placed individually in 10% formalin for two to three weeks, then the optic lenses were carefully removed. After being rinsed with distilled water, the lenses were dried at 80 °C for two days and weighed to the nearest 0.01 mg on an analytic balance (Mettler AE-100). The combined dry weight of both right and left lenses of each individual vole was used for statistical analysis (Table 1).

Table 1. The combined lens weight of the right and left eyes in 65 known-age Smith's red-backed voles, *Eothenomys smithii*.

| Age (days) | Lens weight (mg) | Age (days) | Lens weight (mg) | Age (days) | Lens weight (mg) |
|---|---|---|---|---|---|
| 20 | 1.29 | 140 | 3.16 | 255 | 3.70 |
| 20 | 1.37 | 140 | 3.38 | 255 | 3.80 |
| 20 | 1.37 | 142 | 2.98 | 255 | 3.60 |
| 22 | 1.32 | 160 | 3.28 | 258 | 3.94 |
| 32 | 1.92 | 160 | 3.08 | 282 | 3.80 |
| 32 | 1.84 | 160 | 3.28 | 282 | 3.90 |
| 38 | 1.83 | 160 | 2.85 | 282 | 3.80 |
| 43 | 2.25 | 160 | 3.14 | 300 | 3.81 |
| 44 | 2.37 | 176 | 3.48 | 300 | 4.18 |
| 44 | 2.30 | 180 | 2.92 | 300 | 4.03 |
| 44 | 2.37 | 180 | 3.80 | 350 | 4.22 |
| 50 | 2.24 | 180 | 3.62 | 350 | 4.25 |
| 50 | 2.06 | 180 | 3.43 | 400 | 4.00 |
| 60 | 2.13 | 200 | 3.44 | 468 | 4.71 |
| 70 | 2.59 | 200 | 3.26 | 500 | 4.60 |
| 80 | 2.50 | 200 | 3.00 | 550 | 4.00 |
| 100 | 2.61 | 200 | 3.29 | 550 | 4.37 |
| 105 | 3.24 | 200 | 3.36 | 600 | 4.40 |
| 120 | 2.69 | 200 | 3.74 | 600 | 4.72 |
| 120 | 2.92 | 214 | 3.50 | 600 | 4.44 |
| 140 | 3.02 | 236 | 3.43 | 600 | 4.41 |
| 140 | 3.36 | 250 | 3.78 | | |

**2. Statistical procedure**

The mathematical model for the relationship between lens weight ($W$) and age in days ($A$) pioneered by Hagen *et al.* (1980) and Takada (1982a, 1982b) was used for this study, *i.e.*,

$$W = a + b\log_{10}A \qquad (1)$$

where $a$ and $b$ are parameters to be estimated from the data set. Here, when we define $W = Y$ and $\log_{10}A = X$ in this model, the equation (1) could be $Y = a + bX$, thereby, making linear regression analysis available for the relationship between $Y$ (lens weight) and $X$ (age in days after logarithmic transformation). In the regression analysis, data of $X$ and $Y$ should exhibit homoscedasticity (Dapson 1980, Zar 1984), which we confirmed for our data in accordance with Zar's (1984) procedure.

The linear regression equation $Y = a + bX$ refers to the regression of $Y$ (the dependent variable) on $X$ (the independent variable). When estimating age using dry lens weight, the lens weight ($W$) should be regressed on age ($A$) (Ishii 1975, Hagen *et al.* 1980, Zar 1984). Therefore, a predicted age $\hat{X}_i$ for a given lens weight $Y_i$ and the confidence limits of $\hat{X}_i$ should be calculated on the basis of the procedure known as inverse prediction (Dapson 1980, Zar 1984). Using this procedure, the predicted $\hat{X}_i$ at a given $Y_i$ is given by the equation

$$\hat{X}_i = \frac{Y_i - a}{b},$$

and the confidence limit $L$ ($L_u$, the upper limit ; $L_l$, the lower limit) is calculated from the equation

$$\left.\begin{matrix} L_u \\ L_l \end{matrix}\right\} = \bar{X} + \frac{b(Y_i - \bar{Y})}{K} \pm \frac{t}{K}\sqrt{S^2_{YX}\left[\frac{(Y_i - \bar{Y})^2}{SS_X} + K\left(\frac{1}{m} + \frac{1}{n}\right)\right]}$$

where $\bar{X}$ = the mean of $X$, $\bar{Y}$ = the mean of $Y$, $K = b^2 - t^2 s_b^2$, $s_b$ = the standard error of the regression coefficient, $S^2_{YX}$ = the residual mean square, $SS_X$ = the sum of squared deviations of $X$, $t$ = Student's $t$ ($df = n$-2, $p = 0.05$), and $n$ = sample size (Dapson 1980, Zar 1984). When $m = \infty$, $L$ indicates the confidence limits of the mean prediction for the population, and when $m = 1$, $L$ represents the confidence limits of the individual prediction (Dapson 1980). For the purposes of this study, $X$ and $L$ have been logarithmically transformed, so that $10^{\hat{X}_i}$ gives the predicted age in days and $10^L$ gives its confidence limits.

In this study, data from both males and females were combined for the regression analysis since no significant difference was detected in the slope and elevation of the regression line between males and females. As for the figure showing the regression line with 95% confidence limits (see Fig. 1), we followed the presentation of Hagen *et al.* (1980). Although lens weight was regressed on age, we used the ordinate for the independent variable (age) and the abscissa for the dependent variable (lens weight) because the age was predicted by inverse prediction.

## Results and Discussion

The regression line and its 95% confidence limits for the individual prediction are shown in Fig. 1. The linear regression equation from our data in *Eothenomys smithii* was proved to be

$$W = -1.415 + 2.131 \log_{10} A$$

and therefore a predicted age in days ($\hat{A}$) for a given lens weight in mg ($W$) was given by the equation

$$\hat{A} = 10^{(W+1.415)/2.131}$$

The slope and $Y$ intercept of the regression line, and statistics necessary for calculating the confidence limit are presented in Table 2. Table 2 also includes comparable information from the root vole, *Microtus oeconomus* (Hagen *et al.* 1980), the feral house mouse, *M. m. molossinus* (Takada 1982a) and the large Japanese field mouse, *Apodemus speciosus* (Takada 1982b).

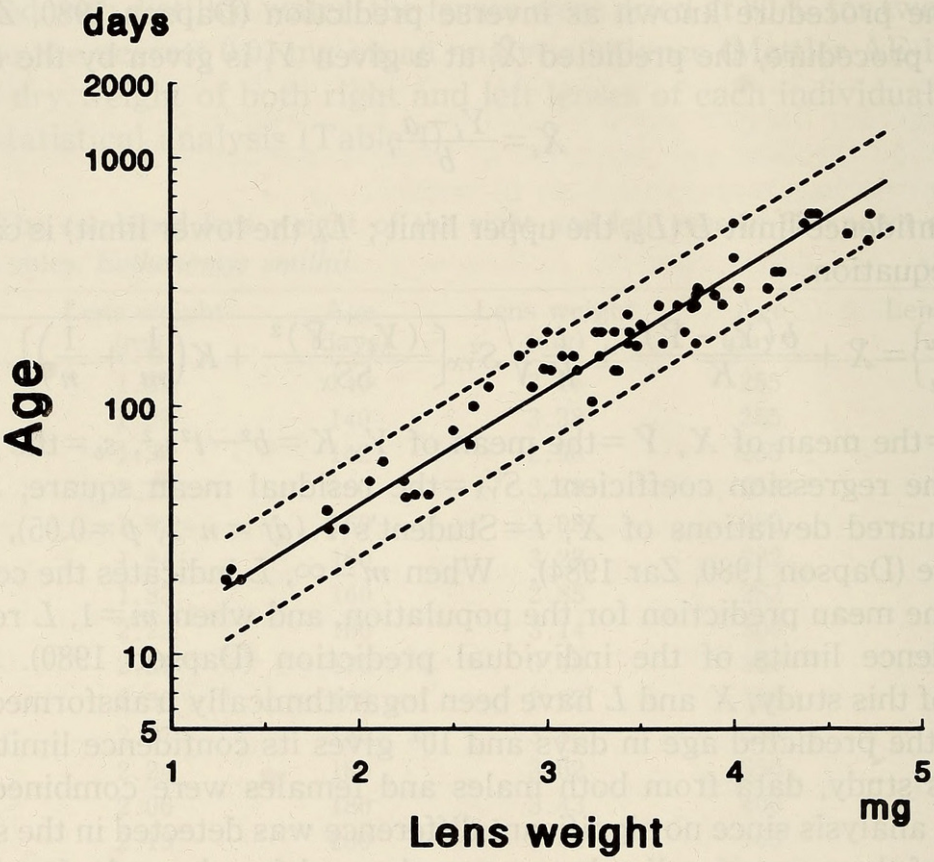


Fig. 1. The relationship between the dry weight of the optic lenses (both the right and left lenses combined) and age in days in 65 known-age Smith's red-backed voles, *Eothenomys smithii*. The solid line indicates the regression line, and broken lines its 95% confidence limits for the individual prediction based on inverse prediction. Note that the vertical axis was used for the independent (age) variable, and horizontal axis for the dependent (lens weight) variable, although lens weight was regressed on age.

Table 2. Statistics required for calculating the predicted ages and 95 % cofidence limits of the Smith's red-backed vole, *Eothenomys smithii*, the root vole, *Microtus oeconomus*, the feral house mouse, *Mus musculus molossinus*, and the large Japanese field mouse, *Apodemus speciosus*.

| species | $n$ | $a$ | $b$ | $r$ | $\bar{X}$ | $\bar{Y}$ | $S_{YX}$ | $S_{YX}/\bar{Y}$ | $SS_X$ | $t$ |
|---|---|---|---|---|---|---|---|---|---|---|
| *E. smithii*<br>This study | 65 | −1.415 | 2.131 | 0.971 | 2.177 | 3.223 | 0.213 | 0.0660 | 10.277 | 1.998 |
| *M. oeconomun*<br>Hagen *et al.* (1980) | 31 | −1.729 | 2.799 | 0.983 | 1.869 | 3.503 | 0.140 | 0.0400 | 2.336 | 2.045 |
| *M. m. molossinus*<br>Takada (1982a) | 73 | −2.954 | 4.13 | 0.976 | 1.994 | 5.281 | 0.358 | 0.0677 | 10.608 | 1.994 |
| *A. speciosus*<br>Takada (1982b) | 14 | −15.474 | 16.122 | 0.988 | 1.867 | 14.625 | 0.867 | 0.0549 | 1.230 | 2.179 |

$n$ : sample size, $a$ : $Y$ intercept, $b$ : slope, $r$ : correlation coefficient, $\bar{X}$ : mean of $X$, $\bar{Y}$ : mean of $Y$, $S_{YX}$ : standard error of estimate, $SS_X$ : sum of squared deviations of $X$, $t$ : Student's $t$ ($d.f.=n-2$, $p=0.05$).

Dapson (1980) and Zar (1984) both recommended the presentation of $S_{YX}/\bar{Y}$ (the standard error of estimate ($S_{YX}$) divided by the mean of $Y$ ($\bar{Y}$)), as an indicator for assessing the fitness of the regression and the accuracy of the technique. Smaller values of $S_{YX}/\bar{Y}$ indicate better fitness of the regression. The value of $S_{YX}/\bar{Y}$ for *E. smithii* (0.0660) is very close to that for *M. m. molossinus* (0.0677) (Takada 1982a), but larger than the values for either *M. oeconomus* (0.0400) (Hagen *et al.* 1980) or *A. speciosus* (0.0549) (Takada 1982b). When studies, which have used the regression analysis for age estimation, are compared (*e.g.*, those on *M. oeconomus* [Hagen *et al.* 1980], *M. m. molossinus* [Takada 1982a], *A. speciosus* [Takada 1982b], *S. floridanus* [Load 1959], *L. californicus* [Connolly *et al.* 1969], *L. arcticus* [Hearn and Mercer 1988] and *L. brachyurus* [Ando *et al.* 1992]), $S_{YX}/\bar{Y}$ is found to range from 0.0400 to 0.0823 (calculated by us). It is accordingly inferred that when the regression line fits the data well, $S_{YX}/\bar{Y}$ may be smaller than *ca.* 0.083 in small to medium-sized mammals such as rodents and lagomorphs. Since the value for *E. smithii* (0.0660) falls within the middle of this range, it can be said that our data from *E. smithii* fit the model equation (1) well.

Confidence intervals also indicate the accuracy of estimates derived from an age determination technique (Dapson 1980). Table 3 shows the predicted age ($\hat{A}$) at a given lens weight ($W$), its 95% confidence limits for the mean prediction and that for the individual prediction in *E. smithii*. The 95% confidence interval for the mean prediction at the mean of $X$ (*i.e.*, $\bar{X}=2.1769$, the predicted age of 150 days) was calculated to be 17 days, occupying 11.3% of the predicted age (150 days). Similar figures have also been obtained for *M. oeconomus* (8.0%, Hagen *et al.* 1980), for *M. m. molossinus* (10%, Takada 1982a) and for *A. speciosus* (15%, Takada 1982b). In *E. smithii*, the 95% confidence interval (144 days) for the individual prediction, at the mean of $X$, occupied 96.0 % of the predicted age (150 days). The corresponding figure for *M. oeconomus* is 48 % (Hagen *et al.* 1980), for *M. m. molossinus* 83 % (Takada

Table 3. 95 percent confidence limits of predicted ages ($\hat{A}$) for the Smith's red-backed vole, *Eothenomys smithii*.

| Lens weight $W$ (mg) | Age $\hat{A}$ (days) | Mean predictions | | Individual predictions | |
|---|---|---|---|---|---|
| | | Lower age limit (days) | Upper age limit (days) | Lower age limit (days) | Upper age limit (days) |
| 1.36 | 20 | 17 | 23 | 12 | 32 |
| 1.73 | 30 | 27 | 33 | 19 | 48 |
| 2.20 | 50 | 46 | 54 | 31 | 80 |
| 2.52 | 70 | 66 | 77 | 45 | 114 |
| 2.85 | 100 | 94 | 106 | 63 | 159 |
| 3.22 | 150 | 142 | 159 | 94 | 238 |
| 3.49 | 200 | 189 | 213 | 126 | 318 |
| 3.86 | 300 | 280 | 323 | 189 | 479 |
| 4.04 | 365 | 338 | 397 | 230 | 584 |
| 4.13 | 400 | 369 | 437 | 252 | 641 |
| 4.33 | 500 | 466 | 563 | 320 | 820 |
| 4.50 | 600 | 544 | 669 | 376 | 967 |

1982a) and for *A. speciosus* 55% (Takada 1982b). The 95% confidence interval for *E. smithii* was similar for the mean prediction, but was slightly broader for the individual prediction, when compared with the three other species. Although the confidence limits are influenced by various factors, such as sample size, the degree of dispersion of data, the mean of $X$ and so on, increasing the sample size may be one possible way to improve the accuracy of age estimation of *E. smithii*.

The combined dry weights of both right and left lenses from 52 wild *E. smithii* captured on Mt. Wakasugi ranged from 1.95 to 4.49 mg (Ando unpublished data). From the equations defined above, a vole with a maximum lens weight of 4.49 mg would be estimated to be 591 days old, with the 95% confidence limits giving a range of 371 to 952 days for the individual prediction. About 80% of the voles (41/52) possessed lenses weighing below 4.04 mg indicating 365 days of a predicted age. Although there have been some field studies on population dynamics of *E. smithii* (Tanaka 1964, Igarashi 1980), no information has been available on the longevity of individuals in the wild, for instance, based on the capture-recapture method. Judging from the existence of voles with lenses weighing over 4.04 mg, it would appear, however, that some individuals in the wild could survive for over one year. In the laboratory, *E. smithii* has been known to live for more than three years (Ando *et al.* 1988). Field studies, in combination with the lens weight technique, are necessary in order to confirm the usefulness of the technique, especially in older voles.

Researchers on rodents have typically used wear of the tooth surface, and the length or the ratio of molar roots to determine age (Abe 1976 for *Clethrionomys rufocanus bedfordiae*, Hikida and Murakami 1980 for *Apodemus speciosus*, Alibhai 1980 for *Clethrionomys glareolus*). These methods, however, can only be employed in rodents which have rooted molars, and Takada (1982a, 1982b) has already demonstrated that even in *M. m. molossinus* and *A. speciosus* which have rooted molars, the lens weight technique may be more reliable than the technique depending on the tooth wear. It should be emphasized, therefore, that the lens weight technique provides the best criterion for assessing age at present, particularly in rodents with rootless molars such as *E. smithii*.

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