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SHORT NOTES

B chromosomes and asymmetry of eye lenses in the yellow-necked mouse, *Apodemus flavicollis* (Rodentia, Mammalia)

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Dry eye lens weight, estimated by method of LORD (1), is one of the best parameters for assessing the age of specimens of rodent species. Due to the absence of blood vessels in the lens, variations caused by physiological and environmental changes are less pronounced than in other (phenotypic) traits. Another important feature is that the weights of the eye lens proceed to increase after attainment of sexual maturity (2). According to DAPSON & IRLAND (3) the accumulation of the soluble fraction of tyrosine in the lens is representative of growth, whereas the conversion of the soluble to the insoluble fraction is regarded as aging. The pace of growth matches the logarithm of age. By measuring the dry lens weight of animals of known age, a growth curve that can be used to estimate the age of specimens can be easily obtained. Such a curve can be of great use in population studies of rodents.

All populations of the yellow-necked mouse, *Apodemus flavicollis,* studied so far are characterized by different B chromosome frequencies. B chromosomes are dispensable supernumerary chromosomes that do not recombine with A chromosomes. B chromosomes evolve independently of the standard chromosome complement (4). The effects of B chromosomes are numerous but it is difficult, with minor exceptions, to follow them at the phenotype level. They differ from one species to another. Developmental effects of B chromosomes are not rare in plants and are occasionally observed in animals. A good example is the grasshopper, *Myrmeleotettix maculatus* (5, 6). In *A. flavicollis* relationships between some morphometric characteristics are changed in the presence of Bs (7).

Measurement of asymmetry of morphometric characteristics is frequently used to study the effects of environmental and genetic factors (8). In this study variations of lens asymmetry relative to the mass of the lenses (i.e. the age of the specimen) were studied in the context of the presence of B chromosomes in populations of *A. flavicollis* with the aim to establish do the presence of Bs influences that relation.

Samples of the yellow-necked mouse *Apodemus flavicollis* were collected from three localities in Yugoslavia. These were Mt. Cer (CQ84), Mt. Avala (DQ64) and Mt. Fruška Gora (DR00) from 1993-1995, and from 1997-2000 (UTM coordinates are in brackets). A total of 133 animals were collected (Cer n=54, Avala n=48, Fruška Gora n=31). Chromosomes were prepared from bone marrow according to the standard procedure (9). The number of chromosomes was defined by scoring 30 metaphases per animal. The number of animals with B chromosomes per population was expressed as a frequency designated as fB. Eyeballs were removed and stored in 10% formaldehyde. After two weeks the lenses were dissected from the eyeballs, cleaned, rinsed with distillated water and dried in an oven at 80ºC for 48 h (10). Immediately after removal from the oven, they were weighed to an accuracy of 0.01 mg using a Mettler laboratory scale. Each lens (l1, l2) of an individual specimen was weighed separately, but without knowledge about left or right origin. The mass of a pair of lenses was used as an age indicator. Weighing of the lenses was performed by the same person (M.V.), with double-checking, without any prior knowledge of the results of chromosome analyses. The differences between pairs of lenses (asymmetry) were calculated as abs(l1-l2). Correlation analysis and Student t-test were performed using Stat 5.0 software.

The frequencies of animals with Bs were similar at the Cer and Avala (0.37 and 0.35) localities, while at Fruška Gora the frequency was higher (0.45), with average frequency 0.38. Dry eye lens weight measurements revealed that the population of specimens from Fruška Gora was the youngest, whereas the population from Avala was the oldest (Table 1). Samples from all three localities were pooled in order to analyze the effects of B chromosomes. Sample of animals with Bs was mostly made of animals with 1B (68.6%) and the rest was made of animals with 2B (19.6%), 3B (9.8%) and 4B (2%). Due to the small number of animals with more than one B we analyzed only the effects of presence of Bs. In pooled samples the ratio of males to females was 1 :1.38. Absolute differences in lens masses ranged from 0.1 to 3.1 mg, representing 0.29-21.65% of the weight of the lens. Estimated measurement error does not exceed significantly the mean value of asymmetry. Taking into consideration

these variations of lens mass, the correlation coefficients between the total weight of both lenses (age) and the absolute asymmetry were calculated for animals with and without B chromosomes (Fig. 1).

TABLE 1.

Weights of dry eye lenses (in mg) ±SE in males (M) and females (F) without (B0) and with (B+) B chromosomes (n – number of animals, fB – frequency of animals with Bs).

Locality	B0			B+			
	F	M	n	F	M	n	fB
Mt. Cer	23.9 ±1.17	24.7 ±1.58	34	24.3 ±1.56	20.9 ±2.19	20	0.37
Mt. Avala	22.7 ±1.80	25.5 ±2.40	31	24.4 ±1.73	26.6 ±2.69	17	0.35
Mt. Fruška Gora	20.4 ±2.39	22.1 ±2.91	17	20.7 ±2.36	20.4 ±1.47	14	0.45
Total	23.5 ±0.80		82	22.4 ±0.80		51	0.38

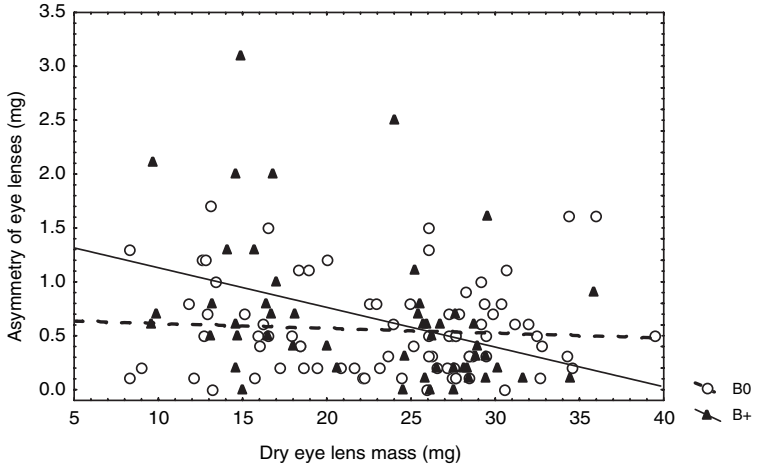

Fig. 1. – Correlation between dry eye lens mass (in mg) and lens asymmetry (abs(l1-l2)) in the group of animals without (B0) and with (B+) B chromosomes

The aim was to see whether the level of asymmetry of the lens was age-dependent in these two groups. It appears that in the group of animals without Bs (B0), the age and asymmetry of eye lenses were not dependent (r=0.08), while in the group of animals with Bs (B+) the correlation was significant and with a negative sign (r=-0.38). Results of t-test (t=-2.88, p=0.006) approve that the correlation coefficient differs significantly from zero. Therefore, in the last group the asymmetry decreased with age. It is possible that B chromosome carriers with a high degree of asymmetry were eliminated from populations at a higher rate. Previously it was shown that the presence of Bs had an effect of prolonging the mitotic cycle in the plant *Lolium perenne* (11). It is possible that the presence of Bs affected early embryonic and postnatal development. GLIWICZ & JANCEWICZ (12) proposed that significant differences in weight between left and right lenses in specimens of *Sorex minutus* are the consequence of illness. It is possible that weight differences between left and right lenses could be ascribed to different diseases and developmental disturbances. If differences in the weights of the lenses lead to anomalies in vision (which is very important for *A. flavicollis* as they are mostly nocturnal animals), it can be expected that selection pressure will be very high in animals that have such disorders. But differences in dry weight between lenses could be also produced by differences in developmental stability between groups of animals with and without Bs.

In the population from Mt. Jastrebac, BLAGOJEVIĆ & VUJOŠEVIĆ (13) found that under conditions of stress due to overcrowding, young animals with Bs were preferentially eliminated. Furthermore, it was found that B chromosomes in this species were characterized by the presence of specific molecular marker sequences (14). The results of this study imply that the presence of B chromosomes influences relation between lens asymmetry and age, although the causative role of Bs remains to be established.

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