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DISTRIBUTION OF B CHROMOSOMES IN AGE CATEGORIES OF THE YELLOW-NECKED MOUSE *APODEMUS FLAVICOLLIS* (MAMMALIA, RODENTIA)

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Abstract — The presence of B chromosomes (Bs) is frequently found in populations of yellow-necked mouse, *Apodemus flavicollis*. Differences in frequencies of Bs in age categories were analyzed in 717 animals in order to clarify the mechanisms of their maintenance in populations of *A. flavicollis*. The absence of statistically significant differences in the frequency of Bs between six age categories indicates that the maintenance of Bs in populations can be explained by in terms of their contribution to overall genetic diversity of the species rather than by parasitic behavior.

Key words: *Apodemus flavicollis*, B chromosomes, age categories

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INTRODUCTION

It is not easy to define B chromosomes (Bs) since this name includes a variety of extra chromosomes which display conspicuous heterogeneity in both their nature and evolutionary dynamics. Dispensability, which means that they are not required for the carrier's development and survival, is the only characteristic shared by all Bs. Additionally, the absence of recombination with members of the basic A chromosome set and non-Mendelian modes of inheritance often characterize Bs (Jones, 1995). The presence of Bs is regularly found in some, but not all, individuals within a population. While it is generally estimated that almost 15% of species possess Bs, their occurrence in mammals is much lower (1.2%), so the genus *Apodemus* is an exception, as six species out of 42 feature the presence of Bs. In the yellow-necked mouse *Apodemus flavicollis*, Bs are found in almost all populations with a wide range of frequencies (Zima and Macholan, 1995; Kartavtseva, 2002; Vujošević and Blagojević, 2004; Wojcik et al., 2004; Rovatsos et al., 2008). The constant maintenance of Bs is a long-lasting subject of debate. It is supposed that a kind of genetic equilibrium is necessary for preservation of B chromosomes in natural populations of many species. This equilibrium could result from accumulation of otherwise deleterious Bs regardless of their number and selection against B carrying individuals (the parasitic model of Östergreen, 1945), or else it might result from beneficial effects of Bs in low numbers but harmful effects in high numbers (the heterotic model of White, 1973). In the last decade, the prevailing opinion has been that Bs are genomic parasites. This has resulted more from the lack of evidence for beneficial effects of Bs than from new proofs for the parasitic model. For this model of maintenance, the existence of an accumulation mechanism is *sine qua non*. To avoid this necessity in species without drive, Camacho et al. (1997) proposed that, as a result of the arms race with A chromosomes, parasitic Bs may not be in equilibrium, but rather passing through successive stages, i.e., B invasion, drive-suppression, and near-neutral extinction with or without regeneration.

If Bs described as parasites reduce mean fitness of individuals carrying them, it may be expected that the frequency of animals with Bs will decrease with age, either abruptly or constantly. With the aim of clarifying mechanisms of B chromosome maintenance in populations of *A. flavicollis*, differences in frequencies of Bs in several age categories were analyzed.

MATERIAL AND METHODS

A total of 717 (360 males, 357 females) yellow-necked mice, *Apodemus flavicollis*, were collected from 12 localities in Serbia (UTM coordinates in brackets): Mt. Jastrebac (EP30), Mt. Cer (CQ84), Ada (CQ82), Mt. Goč (DP82, Mt. Avala (DQ64), Mt. Fruška Gora (DR00), Mt. Maljen (DP28), Košutnjak (DQ55), Titelski Breg (DR40), Devojački Bunar (DQ98), Mt. Beljanica (EP58), and Mt. Tara (DR40). Chromosomes were obtained directly from bone marrow using a standard procedure. Differential chromosome staining, G-banding, and C-banding were carried out according to the methods of Seabright (1971) and Sumner (1972). The presence of B chromosomes was scored by analyzing 30 metaphases per animal. The number of animals with Bs per population was expressed as a frequency designated as fB and the number of Bs per animal as fB/B carrier.

Dry eye lens weight was estimated according to the procedure of Nabaglo and Pachinger (1979) to an accuracy of 0.1 mg with a Mettler laboratory balance and used to assess the age of specimens. Statistical analysis was performed using Statistica 6.0 software.

RESULTS

The animals were grouped into six age categories according to dry eye lens weight (Table 1). This is

Table 1. Distribution of animals without (B0) and with (1B and >1B) B chromosomes in age categories according to dry eye lens weight, frequencies of Bs per B carrier (fB/B), and frequency of animals with Bs (fB).

Lens weight (mg)	0B	1B	>1B	fB/B	fB
≤10	15	5	2	1.43	31.82
11-15	76	16	8	1.50	24.00
16-20	108	24	12	1.36	25.00
21-25	86	17	17	1.53	28.33
26-30	182	46	22	1.44	27.20
>30	60	16	5	1.33	25.93
Total	527	124	66	1.43	26.50

Table 2. Average dry eye lens weight (mg) in animals with (B+) and without (B0) B chromosomes and frequency of animals with Bs at different localities.

Locality	Bs	Lens weight	±SD	N	fB
Mt. Jastrebac	B0	19.80	±6.72	26	16.13
	B+	21.74	±3.82	5	
Mt. Cer	B0	24.25	±6.03	52	35.00
	B+	22.33	±6.73	28	
Ada	B0	21.51	±5.25	90	15.90
	B+	22.72	±4.59	16	
Mt. Goč	B0	22.32	±6.64	11	42.11
	B+	17.41	±6.65	8	
Mt. Avala	B0	22.98	±7.09	144	22.99
	B+	23.57	±6.49	43	
Mt. Fruška Gora	B0	20.55	±8.13	79	30.63
	B+	21.06	±8.12	34	
Mt. Maljen	B0	19.98	±2.83	6	45.00
	B+	22.42	±2.87	9	
Košutnjak	B0	27.70	±3.15	57	16.67
	B+	27.81	±2.30	11	
Titelski Breg	B0	20.91	±5.25	14	6.67
	B+	16.10	±0.00	1	
Devojački Bunar	B0	19.45	±7.05	17	34.42
	B+	19.22	±6.65	9	
Mt. Beljanica	B0	19.00	±7.77	9	43.75
	B+	23.16	±6.00	7	
Mt. Tara	B0	24.48	±5.84	23	43.90
	B+	25.14	±4.43	18	
Total		22.64	±6.66	717	26.33

one of the best parameters for assessing the age of specimens of rodent species. Accumulation of the soluble fraction of tyrosine in the lens and conversion of the soluble to the insoluble fraction is regarded as aging. The tempo of growth matches the logarithm of age. Lens weight (for both lenses) ranged from 7.2 to 39.5 mg, values which correspond to ages of from 1 month to more than 2.5 years. The average frequency of animals with Bs was 0.26, and the average frequency of Bs per B carrier was 1.43. Most B carriers (65.3%) had one B chromosome, 27.4% had 2 Bs, and 6.3% 3 Bs (Table 2). The highest number of four Bs was found in only two animals. No statistically significant difference in the frequency of specimens with Bs was found in successive age groups (2X2 Tables), although the frequency of animals with Bs between localities was significantly different (Table 3) and ranged from 0.067 at Titelski Breg to 0.439 on Tara Mountain. The average age of animals with and without Bs did not differ significantly inside age groups. The localities did not differ in the age of specimens except for Košutnjak, where animals were significantly older on average than at the other eight localities (Table 2).

DISCUSSION

Chromosomes of the B type are described as parasites because they provide for their own reproduction at the expense of the carrier, thus reducing its fitness. The absence of significant differences in frequencies of Bs between the six age categories, together with our earlier findings, indicates that the parasitic model is not operating in *A. flavicollis*. The heterotic mode of B chromosome maintenance better explains the constant maintenance of Bs in *A. flavicollis* populations, but with the observation that the effects of Bs in this species are more pronounced at the population level.

Other findings about B chromosomes in *Apodemus flavicollis* that support the idea that Bs in this species do not behave as genome parasites are the following. Firstly, no mechanism of accumulation of Bs has yet been observed in this species. The same frequency of Bs was found in bone marrow and testicular tissue, indicating that there is no preferential segregation in males, while the frequency in females is always the same as in males (Vujošević et al., 1989, and unpublished data). Populations of species having several generations

Table 3. Differences in frequencies of animals with Bs among localities (Chi-square values).

	Mt. Cer	Ada	Mt. Goč	Mt. Avala	Mt. Fruška Gora	Mt. Maljen	Košutnjak	Titelski Breg	Devojački Bunar	Mt. Beljanica	Mt. Tara
Mt. Jastrebac	**3.81***	0.02	**4.13***	0.73	2.40	**5.09***	0.00	0.80	2.61	**4.23***	**6.26****
Mt. Cer		**10.00*****	0.33	**4.14***	0.52	0.69	**5.83***	**4.78***	0.00	0.44	0.91
Ada			**7.58****	2.63	**6.98****	**9.46****	0.07	0.77	**5.18***	**7.46****	**13.80*****
Mt. Goč				3.38	1.08	0.03	**5.31***	**5.41***	0.26	0.01	0.02
Mt. Avala					1.86	**4.65***	1.08	2.17	1.67	3.42	**7.50****
Mt. Fruška Gora						1.73	**3.72***	3.66	0.20	1.21	2.57
Mt. Maljen							**6.65****	**6.17****	0.51	0.01	0.01
Košutnjak								0.96	3.40	**5.34***	**9.02*****
Titelski Breg									**4.03***	**5.56***	**6.79****
Devojački Bunar										0.35	0.57
Mt. Beljanica											0.00

Statistically significant x^2 values are in bold (* p≤0.05, ** p≤ 0.01, ***p≤ 0.005).

per year, such as *A. flavicollis*, are characterized by a kind of imperfect equilibrium (White, 1973). This means that although the frequency of specimens with Bs fluctuates seasonally during the year, it stays stable from year to year. For instance, the frequency of *A. flavicollis* individuals with Bs did not change significantly over a period fo eight years regardless of great changes in abundance (Vujošević, 1992, and unpublished data). Stability in the frequency of animals with Bs in successive years was also reported by Zima and Macholan (1995). On the other hand, it was found that seasonal changes in the frequency of animals with Bs can be significant under conditions of stress produced by competition resulting from overcrowding (Blagojević and Vujošević, 1995). When population density is moderate, competition for food and space is stable, thus producing equilibrium in the frequency of animals with Bs throughout the year (Vujošević and Blagojević, 1995). A possible heterotic effect of B chromosomes on body mass was found by Zima et al. (2003). Analyses of nonmetric traits (Blagojević and Vujošević, 2004) point to population density as a significant factor influencing the observed variation in the frequency of specimens with Bs and developmental homeostasis at the same time. According to predictions of the parasitic model the highest frequency of Bs would be expected at those localities with more favorable habitats, but Vujošević et al. (2007) found the opposite to be true in *A. flavicollis*, as deduced by estimating habitat quality from the index of overall body size.

The long-term presence of Bs in populations could be explained by their influence on the genetic variability of species possessing them (Blagojević and Vujošević, 2004; Wójcik et al., 2004). Increased variability offers species a greater chance to survive in changeable environmental situations. Populations of *Apodemus flavicollis* inhabiting areas with more extreme climatic conditions showed an increased frequency of animals with Bs (Vujošević and Blagojević, 2000). Zima and Macholan (1995) observed a clinal trend of increase in B chromosome frequencies from Central Europe, with the exception of some populations from Poland (Wójcik et al., 2004). Tokarskaia et al. (2000) found a positive correlation between the presence of Bs and heterozygosity for RAPD loci in populations of Siberian roe deer, *Capreolus pygargus*, which indicates a contribution by Bs to genetic variation of the species. There are a number of convincing reports indicating that Bs enhance the fitness of plant carriers (Hutchinson, 1975; Teoh et al., 1976; Teoh and Jones, 1978). A well-documented instance where Bs appear to be associated with selectively advantageous effects in the case of *Allium schoenoprasum* (Bougourd and Parker, 1975, 1979a, 1979b). Our results, together with the fact that the number of species without mechanisms of B accumulation is not small, indicate that maintenance of Bs in populations could be explained by their contribution to the overall genetic diversity of species.

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ДИСТРИБУЦИЈА B ХРОМОЗОМА У УЗРАСНИМ КАТЕГОРИЈАМА ЖУТОГРЛОГ МИША, *APODEMUS FLAVICOLLIS* (MAMMALIA, RODENTIA)

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Прекобројни хромозоми називају се B хромозоми ако задовољавају три критеријума: нису неопходни за преживљавање, не спарују се са хромозомима А сета и не наслеђују се менделовски. B хромозоми су врло често присутни у популацијама жутогрлог миша, *Apodemus flavicollis*. Њихово одржавање у популацијама објашњава се са два супростављена модела. Модел означен као паразитски тврди да се B хромозоми одржавају захваљујући механизму акумулације упркос штетним ефектима на фитнес носилаца. С друге стране, хетеротички модел сугерише да у одсу-

ству акумулације мали број B хромозома има адаптивну предност за носиоце, док већи број може бити штетан. Ако B хромозоми, који су означени као паразитски, редукују фитнес код јединки које су њихови носиоци, онда је очекивано да фреквенца јединки са B хромозомима опада са старошћу. Анализирана је разлика у фреквенци B хромозома у узрасним категорија-ма код 717 јединки са циљем да се разјасне механизми њиховог одржавања у популацијама *A. flavicollis*. Одсуство статистички значајних разлика у фреквенци B хромозома између 6 узрасних категорија указује да се одржавање B хромозома у популацијама ове врсте може објаснити њиховим доприносом укупној генетичкој разноврсности пре него паразитским понашањем.