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B-chromosomes behaviour during meiosis of yellow-necked mouse, *Apodemus flavicollis*

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A b s t r a c t . The behaviour of B-chromosomes in meiosis was studied in 44 males of yellow-necked mouse, *Apodemus flavicollis*. The Bs behave in a non-Mendelian fashion during meiosis I i.e., appear as univalents, regardless of the number of Bs, and segregate in a random fashion. The meiotic drag of Bs, resulting from Bs lagging and premature division of Bs into chromatids, was found in 1B animals. The Bs drag amounts to about 6% of cells and is not substantial; however due to dispensable nature of Bs, it has to be compensated through drive in females or heterotic effects of Bs. This result gives support for the heterotic model of Bs evolution in *A. flavicollis*. On the other hand, the number of chiasmata on A-chromosomes showed increasing tendency with the number of Bs in the karyotype. As the chiasma effect is characteristic for parasitic Bs, it gives support for the parasitic model of Bs evolution. With contrasting results, the hypothesis of a combined model of Bs evolution, in which the fitness of B-bearers changes around the year or in different environmental conditions is discussed.

Key words: genome parasites, heterotic Bs, meiotic drag, nondisjunction

Introduction

B-chromosomes (Bs) are optional extra elements in the nuclear genome and they are additional to the basic complement of the A-chromosomes. Bs constitute a very variable class of chromosomes in respect of morphology, modes of transmission, effects and population dynamics. The only trait characteristic to all of them is the fact that they are not necessary for survival, i.e. normal processes of growth and development of the individuals carrying them (J o n e s & R e e s 1982).

B-chromosomes are rather rare in mammals, at least in comparison with their frequent occurrence in insects and plants (J o n e s & R e e s 1982). Until now, Bs have been found in 55 mammalian species, mainly rodents (V u j o š e v i ć & B l a g o j e v i ć 2004). Among them there are six species of *Apodemus*, with two species, *Apodemus penninsulae* (Korean field mouse) and *Apodemus flavicollis* (yellow-necked mouse) showing common and frequent occurrence of B-chromosomes (Z i m a & M a c h o l á n 1995). In populations of *A. flavicollis* B-carrying mice consist on average 50% of individuals. *A. flavicollis* is therefore a suitable model for studying B-chromosome dynamics in mammals, as it is a widespread and common species with high frequencies of B-carrying individuals in most natural populations.

The frequencies of Bs in a single *A. flavicollis* population are fairly stable over long periods of time (V u j o š e v i ć 1992, Z i m a et al. 2003). As the Bs are dispensable in nature, to avoid elimination through random genetic drift or natural selection, they require special combinations of evolutionary forces promoting them in populations (C a m a c h o

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et al. 2000). The Bs with negative influence on their hosts would be eliminated by natural selection. Elimination, although caused by random forces, would also be the fate of neutral Bs. Both categories of Bs therefore require accumulation mechanisms, i.e. transmission rates greater than Mendelian 0.5, to persist in populations and create the systems of balanced polymorphisms. Bs with accumulation mechanisms, i.e. driving Bs, are considered parasites and the model of their evolution is known as parasitic or selfish (Ostergren 1945, Camacho et al. 2000). In contrast, the Bs may have beneficial effects on their carriers and in such cases they would be maintained in the population without drive, as the B-carriers would be selectively advantageous to individuals without Bs. Infinite accumulation of such Bs would be prevented through reduced fitness of individuals with high numbers of Bs or meiotic drag of Bs, i.e. losses of B-chromosomes during gamete production in B-bearers. This model of Bs evolution and maintenance is called heterotic (White 1973, Camacho et al. 2000). The mechanism of Bs maintenance in yellow-necked mice populations is still debatable, with both parasitic and heterotic models being possible in geographically different populations (Wójcik et al. 2004).

The Bs in *A. flavicollis* could be genome parasites. The negative influence of Bs would be apparent in conditions of an overcrowded population, where the young mice with Bs would be eliminated. Such a scenario was suggested on the basis of negative correlation between the population density and frequency of B-carrying immature animals found in the overcrowded population of *A. flavicollis* (Blagojević & Vujošević 1995). However, in the parasitic model negative influence of Bs has to be balanced by the mechanism of accumulation, which so far has not been found in yellow-necked mice. There are two general ways of Bs accumulation in animals: premeiotic and meiotic mechanisms (Jones 1991). The premeiotic mechanism is connected with mitotic instability of Bs and their preferential segregation in the cells of the germ line. The meiotic mechanism is characteristic for oogenesis, where B chromosomes may segregate preferentially to the secondary oocyte. In yellow-necked mouse a premeiotic mechanism of Bs accumulation was not found (Vujošević et al. 1989). However, the existence of the meiotic mechanism still cannot be overruled as nothing is known about female meiosis in this species.

On the other hand, the heterotic model of Bs maintenance in *A. flavicollis* populations found support in the research work of Zima et al. (2003), who observed a positive correlation between the body mass in males and the number of Bs in the individual karyotype. B-chromosomes may positively influence the growth rate and thus bigger animals may have higher chances of winter survival. The hypothesis found support in geographical patterns of B-chromosome distribution in the species range (Zima et al. 2003). First, the frequencies of B-chromosome individuals positively correlated with altitude and the average number of subzero temperature days, i.e. more extreme climatic conditions (Vujošević & Blagojević 2000). Second, an increase in body size from southern to northern Europe in *A. flavicollis* is in agreement with the latitudinal increase of B-chromosome frequency (Zima & Macholán 1995). Fairly constant frequencies of B-chromosomes in a population would be maintained by the balance between the positive fitness effects at the level of individuals and the elimination of Bs at the level of gametes (Camacho et al. 2000, Zima et al. 2003).

A better understanding of B-chromosome dynamics in the populations of *A. flavicollis* would be possible with more data on the transmission and inheritance of Bs in this species. In this paper we analyze the course of I meiotic division in males to give more detailed information on Bs transmission in yellow-necked mice. The basic information about Bs configurations at the prophase I in *A. flavicollis* was given by Vujošević et al. (1989).

The Bs appear as univalents or bivalents at prophase I, they never form multivalents or attach themselves to any of A-chromosomes (V u j o š e v i ć et al. 1989). In this paper we wish to consider two particular aspects. First, we give more detailed information on the pairing rate of Bs and the behaviour of B univalents at the prophase I and describe the mode of Bs segregation during the I meiotic division. These data allow one to estimate the level of possible losses of B-chromosomes during male meiosis of *A. flavicollis*. Second, we estimate the number of chiasmata on the A-chromosomes to check the so-called chiasma effect of B-chromosomes. The knowledge of the level of B-chromosomes drag and possible chiasma effect allows one to discuss the combinations of evolutionary forces responsible for the maintenance of Bs in populations of *A. flavicollis*.

Material and Methods

In total, 44 males, born and brought up in captivity, were used for the study. The animals of the parental generation were collected from natural population in the vicinity of Białystok (23º15' E, 53º13' N), Poland. Mitotic preparations were done by the standard method from the bone marrow and stained conventionally with Giemsa reagent. Any animal with the chromosome number higher then 48 standard for *A. flavicollis* was considered to bear a B-chromosome. The number of chromosomes was confirmed on the meiotic preparations, where mitotic spreads originated from spermatogonia. Meiotic preparations were done by the method of E v a n s et al. (1964) modified by V u j o š e v i ć et al. (1989). In each individual diakinesis/MI spreads were scored to determine the number of conjugating elements and behaviour of B-chromosomes at the I prophase. Late diploten/diakinesis spreads were used for chiasma counts in A-chromosomes. Metaphase II spreads were used for estimation of mode of Bs segregation during the I meiotic division and additionally for calculation of anaphase I nondisjunction of A-chromosomes.

The differences in the numbers of diakinesis spreads with different numbers of elements were compared with a Chi-square test for homogeneity. The difference in numbers of chiasmata was tested by non-parametric analysis of variance (the Kruskal-Wallis test) at $P = 0.05$.

Results

In total, 33 out of 44 males had B-chromosomes in their karyotype (Table 1). Twenty seven of them had 1B (49 chromosomes), five – 2Bs (50 chromosomes) and only one – three B-chromosomes (51chromosomes). The number of chromosomes in the karyotype was confirmed by spermatogonia mitotic spreads in 17 males (three with 0B and 14 with 1B) and always the same number of Bs was found. The Bs were similar in size to the smallest A-chromosome pairs.

Table 1. The karyotypes of *A. flavicollis* males used in this study with respect to B numbers. D – the expected number of elements at diakinesis/MI spreads after V u j o š e v i ć et al. (1989).

Karyotypic class	Diploid number of chromosomes (2N)	Number of individuals	D
0B	48	11	24
1B	49	27	25
2B	50	5	25 or 26
3B	51	1	26

Animals without B-chromosomes (0B karyotypic class) formed 23 autosomal bivalents and one bivalent of sex chromosomes at diakinesis/MI spreads. No spreads with a higher number of elements and hence univalents originating from A-chromosomes were found (Table 2). Animals with one B-chromosome formed 24 bivalents and a single B-chromosome appeared as univalent (Fig. 1a).The Bs did not associate with A-chromosomes. In 1B individuals we found nine spreads with B-chromosome divided prematurely into chromatids (Table 2, Fig. 1b). As altogether 649 diakinesis/MI spreads were scored in 1B individuals, the premature division of B-chromosome occurred in 1.4% of spreads. The number of elements on the diakinesis spreads in animals with two Bs was 25 or 26 with bivalent of Bs or two univalents respectively (Fig. 1c,d). The spreads with B-chromosome bivalent were more frequent than the spreads with univalent B-chromosomes (Table 2), which could suggest that proportionally smaller numbers of cells were prone to non-Mendelian segregation. We did not find any prematurely divided Bs in the animals with two B-chromosomes. In animal with three Bs, the numbers of scored spreads were too low to allow for any comparisons of frequency of spreads, hence we could only say that two configurations were present – bivalent and univalent or three univalents of B-chromosomes (Table 2). Multivalent configurations were not found. All the meiotic configurations were in agreement with

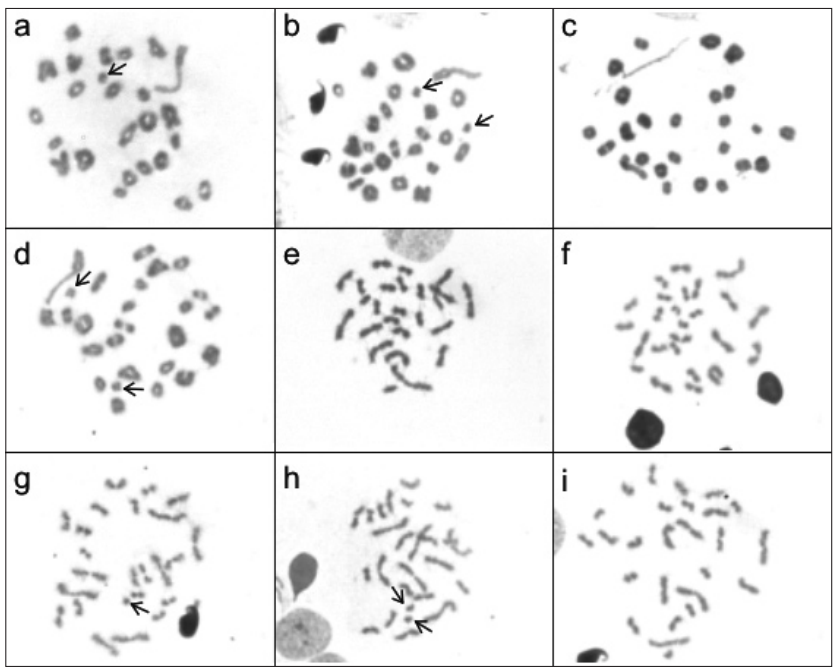

Fig. 1. Meiotic stages of diakinesis/MI and metaphase II in *A. flavicollis* males with different numbers of B-chromosomes. The arrows indicate B univalents (a), (d) or B-chromatids (b), (g), (h). a) Diakinesis/MI with 25 elements including B univalent (1B male, 2N = 49 chromosomes); b) Diakinesis/MI with 26 elements including two B-chromatids (1B male, 2N = 49 chromosomes); c) Diakinesis/MI with 25 elements including Bs bivalent (2B male, 2N = 50 chromosomes); d) Diakinesis/MI with 26 elements including Bs univalents (2B male, 2N = 50 chromosomes); e) Metaphase II with standard 24 chromosomes (0B male, 2N = 48 chromosomes); f) Metaphase II with 25 chromosomes and 1B chromosome (1B male, 2N = 49 chromosomes); g) Metaphase II with 25 elements, i.e. 24 chromosomes and one B-chromatid (1B male, 2N = 49 chromosomes); h) Metaphase II with 26 elements, i.e. 24 chromosomes and two B-chromatids (1B male, 2N = 49 chromosomes); i) Metaphase II with 26 chromosomes and 2B chromosomes (2B male, 2N = 50 chromosomes).

observations of V u j o š e v i ć et al. (1989), except three univalents configuration in 3B males which was not observed by these authors.

Table 2. The number of elements at diakinesis/MI spreads in *A. flavicollis* males with different number of Bs. uni – univalent Bs, biv – conjugated Bs.

Karyotypic class	*n*	Number of spreads	Number of spreads with the number of elements (Bs configuration)			
			24	25	26	27
0B	11	169	169	-	-	-
1B	27	649	-	640 (uni)	9*	-
2B	5	151	-	113 (biv)	38 (uni)	-
3B	1	8	-	-	4 (biv + uni)	4 (uni)

* spreads with B-chromatids resulting from precocious division of B-chromosome

Chiasmata were counted in 18 individuals (Table 3). The number of chiasmata varied from 25 to 31 with an overall mean of 28.5 chiasmata per spread. Longer chromosomes had often two or three chiasmata, while shorter ones usually one chiasma and they appeared in a cross-configuration. In smaller chromosomes the chiasmata could sometimes not be seen clearly. However, as the chromosomes were properly paired, we assumed that the chiasma terminalized almost completely and was replaced with an end-to-end association of homologous chromatids. In an individual with 2Bs, if Bs formed a bivalent, it appeared in a cross-configuration the same as that of small acrocentrics of standard karyotype, which meant that B-chromosomes properly formed one chiasma. Chiasma formed by Bs was not included in the overall number of chiasmata in this individual as the comparisons of chiasma counts concerned A-chromosomes only. The numbers of chiasmata were compared between the mice with 0B, 1B, and 2Bs in the karyotype. The difference was not statistically significant ($H_{(2, N = 46)} = 3.27$, $P = 0.19$), however, the trend of increasing number of chiasmata on A-chromosomes bivalents with the increasing number of B-chromosomes could be noted (Table 3).

Table 3. Number of chiasmata on A-chromosomes at late diploten/diakinesis spreads from *A. flavicollis* males with different number of Bs.

Karyotypic class	n	Number of spreads	Mean ± SE	Increase in number of chiasmata	Min-Max
0B	6	12	27.9 ± 1.7	0.00	25-31
1B	11	30	28.6 ± 1.4	0.71	26-31
2B	1	4	29.5 ± 1.7	1.58	27-31
Total	18	46	28.5 ± 1.6	-	25-31

Bs segregation was estimated from metaphase II spreads. In 0B mice the metaphase II spreads with a proper number of 24 chromosomes after reduction were the most frequent (Table 4, Fig. 1e). The spreads with lower numbers of chromosomes were most probably the effect of preparation and no spreads with a higher number of chromosomes indicating the nondisjunction of A-chromosomes were found. However, on the assumption of one hyperploid spread (and one hypoploid being its counterpart) in 145 counted spreads, the nondisjunction frequency could be calculated as lower than 1.38%. A low frequency of A-chromosomes nondisjunction, estimated in mice without Bs, allowed for interpretation of spreads in animals with B-chromosomes. The spreads with 25 or 26 chromosomes most probably bore a B-

chromosome rather than the result of A-chromosomes nondisjunction. However, the incidence of A-chromosomes nondisjunction in animals with Bs could not be excluded.

In mice with 1B the numbers of spreads with 24 and 25 chromosomes did not differ significantly from the expected 1 : 1 ratio ($\chi^2 = 2.04$, df = 1, $0.2 > P > 0.1$), although there was some excess of spreads without Bs (Table 4, Fig. 1f). Moreover we found 14 spreads with 25 elements with a single B-chromatid, instead of B-chromosome (Fig. 1g), and two spreads with 26 elements, where two additional elements were B-chromatids (Fig. 1h). These spreads are the result of segregation of a prematurely divided B-chromosome. Although B-chromatids apparently go successfully through I meiotic division, as they can be observed at MII, it is most probable that they would be lost during II anaphase and cells bearing them would be included in the pool of gametes without Bs. If we compare the numbers of 25 chromosome MII spreads with B-chromosome (n = 141) and pooled numbers of 24 chromosome spreads and 25 and 26 elements spreads with B-chromatid (n = 182), we notice that the observed ratio deviates significantly from the expected Mendelian 1 : 1 ($\chi^2 = 5.204$, df = 1, $P < 0.05$).

In 2B mice spreads with 24, 25 and 26 chromosomes were found (Table 4, Fig. 1i). Spreads with 25 chromosomes are most probably the result of normal Mendelian segregation of two B-chromosomes, while both 24 and 26 are the result of two Bs segregating to one pole. As the numbers of 25 chromosome spreads are almost equal to the sum of 24 and 26 spreads (Table 4), it can be assumed that the frequency of cells undergoing Mendelian and non-Mendelian segregation is the same. In a single male with 3 Bs we found only 6 spreads with one or two B-chromosomes after reduction (Table 4). The numbers of spreads are too low for any conclusions about mode of segregation of three B-chromosomes.

Table 4. Metaphase II counts for *A. flavicollis* males with different number of Bs. Standard haploid number of chromosomes in *A. flavicollis* is 24. Spreads with lower numbers are most probably artefacts resulting from preparation, spreads with higher numbers contain B-chromosomes or chromatids.

Karyotypic class	*n*	Number of spreads with haploid number of chromosomes						
		<22	22	23	24	25	26	Total
0B	11	9	2	11	123	-	-	145
1B	27	6	8	17	166	141(14)*	2	340
2B	5	12	3	8	16	32	15	86
3B	1	-	-	1	-	3	3	7

* 14 spreads out of total 155 with 25 elements had B-chromatid instead of B-chromosome

Discussion

The behaviour of B-chromosomes during meiotic division varies from one species to another with numerous examples of different amounts of elimination or accumulation of Bs (J o n e s & R e e s 1982). In consequence, no generalizations can be made about the fate of Bs during meiosis in a particular species. In general, the Bs behave in a non-Mendelian fashion with the result of drive or drag during meiosis. However, in animals, meiotic drive takes place in oogenesis only, where nonsymmetrical division may result in an accumulation of Bs in gametes. In spermatogenesis nondisjunction is not the immediate way of Bs accumulation, as the meiotic division is symmetrical and any gamete with a higher number of Bs has its counterpart in gamete without Bs. Hence, the fate of Bs during spermatogenesis may be discussed rather in the context of elimination of Bs, and whether it is substantial or negligible.

Elimination of Bs during meiotic division in general depends on the non-pairing rate, i.e. the occurrence of Bs as univalents and their behaviour during I meiotic division. Univalent Bs may lag in their movement to the metaphase plate at the first meiotic division to such an extent that they are lost as micronuclei, as happens for example, in wild diploid wheat *Aegilops speltoides* (Mendelson & Zohary 1972). Additionally, the centromeres of B-chromosomes may divide during anaphase I to permit the separation of daughter chromatids, which are incapable of division at anaphase II and are lost as micronuclei. Some exceptions, of course, occur from this generalization. For example, in the grass *Festuca pratensis* B-chromatids lag at the second anaphase but usually are able to reach the second telophase nuclei (Bosemark 1954). However, in general, a crucial factor allowing Bs to avoid elimination during meiosis is firstly, not to appear as univalents, and secondly, if they are univalents, to remain undivided at the first division.

Univalent Bs are unavoidable if the animal is bearing only one B-chromosome. In some species the Bs use other chromosomes to orientate themselves properly during the first metaphase, and for example in grasshoppers it is usually the X chromosome (Fontana & Vickery 1973). In *A. flavicollis* Bs never attach themselves to any of the A-chromosomes (Vujošević et al. 1989, this paper), hence they invariably appear as univalents in animals with one B (Table 2, Fig.1a).

In 2B individuals, bivalent formation at metaphase I usually ensures a correct segregation and high B transmission rate. However, pairing of B-chromosomes is another very variable trait and a full spectrum of variation can be listed: from species with non-pairing Bs to those where the frequency of cells with bivalents is about 50%, as in mottled grasshopper *Myrmeleotettix maculatus*, up to for example 99% of cells with B-bivalents in migratory locust *Locusta migratoria* (Jones & Rees 1982). In *A. flavicollis* two Bs may form a bivalent or remain as univalents (Vujošević et al. 1989, this paper Fig. 1c, d). However we found that bivalent configuration was more frequent and occurred in about 75% of cells (Table 2). The Bs in bivalent formed a chiasma and appeared in the same cross-configuration as the smallest acrocentrics of the A-chromosome set.

Univalent Bs always occur also in 3B animals, as in *A. flavicollis* B-chromosomes never form multivalent configurations (Vujošević et al. 1989, this paper). Hence in 3B males Bs appeared as one bivalent and one univalent or as three univalents (Table 2). Unfortunately because of a small number of metaphase II spreads in only one male with 3B-chromosomes (Table 4), we were not able to estimate the fate of univalents in this karyotypic class. However, we suppose that the dynamics of B-chromosomes in most of the populations of yellow-necked mice depends mostly on 1B and 2B animals, as individuals with three or more Bs are usually quite rare. In the population from which the studied males originated, the mean frequency of animals with more than two Bs counted over six years is about 6% (Banaszek, unpubl. data). In populations from the former Yugoslavia, about 95% of B-bearing animals are mice with one and two Bs (estimated from Blagojević & Vujošević 1995 and Vujošević & Blagojević 1995). The exception is the Filipov population from northern Bohemia where mice with three or more Bs comprise 37% of the collected material and the frequencies of B-carrying animals are the highest recorded for *A. flavicollis* up to now (Zima et al. 2003).

Generally, univalent Bs appear at prophase I in all karyotypic classes of B-carrying animals, although in 2B males the bivalent configuration prevails (Table 2). In most cells of 1B animals univalent Bs move undivided to daughter nuclei, which results in metaphase II spreads with 25 chromosomes and their counterparts with 24 chromosomes (Table 4, Fig. 1f).

Some excess of spreads without Bs (Table 4) suggests some loss due to univalent lagging. In 1.4% of cells univalent B separates prematurely into chromatids (Table 2, Fig. 1b), which segregate to daughter nuclei giving 25 element metaphase II spreads and sometimes they move to one pole resulting in 26 element spreads (Table 4, Fig. 1g, h). As the most probable fate of prematurely separated chromatids is lagging and elimination, the cells bearing them will increase the pool of 0B gametes. Hence, the loss of B-chromosomes takes place at meiosis of 1B males, due to univalents lagging and precocious division of univalent B-chromosomes. The amount of B-chromosomes meiotic drag is not substantial and concerns about 6% of cells at MII stage. However, it has to be remembered that any amount of drive or drag influences the population dynamics of B-chromosomes.

Precocious B division was not observed in 2B males, although univalent Bs were found in about 25% of diakinesis spreads (Table 2, Fig. 1d). It is possible that, although not conjugated, the Bs use each other for the proper orientation on the spindle in a similar way as observed in *A. speltoides* (Mendelson & Zohary 1972). Such behaviour may protect them from the premature segregation into chromatids. Hence, the transmission of B-chromosomes through 2B males most probably goes almost without loss. On the other hand, pairing or using each other for the proper orientation in the first metaphase plate does not assure the Mendelian segregation of Bs in *A. flavicollis*. In *A. speltoides* the segregation of Bs in 2B plants is almost as regular as that of A-chromosomes and results in the presence of one B-chromosome in almost all pollen grains (Mendelson & Zohary 1972). In *A. flavicollis* we found 1 : 2 : 1 ratio of metaphase II spreads with 24, 25 and 26 chromosomes (Table 4), suggesting that in about 50% of cells the Bs segregate properly in a Mendelian fashion, resulting in 25 chromosome spreads, while in the other half both Bs go to the same pole giving in effect 24 and 26 MII spreads (Fig. 1i). As at diakinesis about 75% of cells had conjugated Bs (Table 2, Fig. 1c), it seems that the Bs may segregate to the same pole irrespective of pairing. Such a manner of segregation causes an increase in the number of gametes without Bs. If all Bs in 2B animals segregate in a Mendelian fashion, then these individuals would produce only 1B gametes, while in an observed mode of segregation they may produce 2B, 1B and 0B gametes.

The cells at the stage of metaphase II are not yet gametes and the ratios of the produced gametes may be changed through nondisjunction in the II meiotic division. For example in 1B individuals the gametes with 2Bs may probably appear through II division nondisjunction. It has to be stressed, however, that each case of production of 2B gamete, gives in consequence, an increase in the general ratio of gametes without B-chromosome.

In general, both 1B and 2B animals produce gametes without Bs and we found that some losses of Bs may occur in 1B animals, which increased the pool of 0B gametes. The loss of Bs through meiotic drag, even at a very low level, must lead to the elimination of Bs from any population, if it is not compensated (Camacho et al. 2000). Hence, the drag found at meiosis in *A. flavicollis* males has to be balanced by drive in females or heterotic effects on B-carrying individuals. Such a positive relationship was found between the number of B-chromosomes and body mass in males (Zima et al. 2003). Higher body mass, achieved most probably through higher growth rate, could be beneficial due to increased survival rate during winter (Zima et al. 2003). Our results, showing some level of B-chromosome drag at male meiosis give support for the hypothesis of heterotic effects of B-chromosomes in *A. flavicollis,* as they are in agreement with the opinion of Zima et al. (2003), who suggested that the B-chromosome system in *A. flavicollis* could be included in the ninth category of Camacho et al. (2000). The B-chromosome polymorphism in this category is maintained by equilibrium between evolutionary forces of meiotic drag and the positive effects on the host.

On the other hand, as nothing is known about the behaviour of Bs at female meiosis in *A. flavicollis*, some amount of drive through females still cannot be excluded. Such a scenario was described in *M. maculatus,* where the accumulation of Bs went through female gametes and significant loss of Bs occurred at male gametogenesis (H e w i t t 1973).

Meiotic drive is characteristic for parasitic Bs that are eliminated from populations due to harmful effects on carriers and they need mechanisms allowing them to persist in populations. Parasitic Bs and host A-chromosomes are in a constant arms race over the rate of Bs transmission, with Bs trying to drive as much as possible and A-chromosomes trying to suppress drive and neutralize B-chromosomes (C a m a c h o et al. 1997). One of the mechanisms of A-chromosomes defence is an increase in chiasma frequency in B-bearing individuals, resulting in recombinant progeny, some of which would be resistant to B-chromosome drive and effects. Such a mechanism is called inducible recombination (B e l l & B u r t 1990) and it found empirical support in the clover grasshopper *Eyprepocnemis plorans* B-chromosome system (C a m a c h o et al. 2002). On the other hand, the phenomenon called the chiasma effect of B-chromosomes was long known and described in several species of Orthoptera and also in many plant species (J o n e s & R e e s 1982). However it was usually considered as an adaptive effect of heterotic Bs and not as A-chromosome defence against parasitic Bs.

In *A. flavicollis* we found the increasing trend of the mean number of chiasmata on A-chromosomes with the number of Bs, although the difference in chiasmata numbers between karyotypic classes was not statistically significant (Table 3). Still, the increase in chiasma numbers was 0.7 in 1B males and almost 1.6 in 2B animal in comparison with 0B ones (Table 3). As the chiasma frequency in *A. flavicollis* increases in the presence of Bs, it can be expected that they are to some extent driving parasites. The intensity of chiasma frequency increase depends on the evolutionary status of B-chromosome polymorphism, i.e. drive neutralization by A-chromosomes is accompanied by a weakening of chiasma effect (C a m a c h o et al. 2002). The pattern of chiasma increase in *A. flavicollis* is similar to the effects of a partially neutralized B-chromosome of Moroccan population of *E. plorans* (C a m a c h o et al. 2002). Hence, it can be suggested that the *A. flavicollis* B-chromosome in the studied population is a parasite in the process of neutralization.

As was stressed by Z i m a et al. (2003), due to the varying effects of B-chromosomes depending on the evolutionary status of a given B-chromosome, it is not possible to give generalized answers for B-chromosome dynamics in the whole species. However, our findings of meiotic drag in male meiosis give support for the hypothesis of heterotic effects of Bs in *A. flavicollis* (Z i m a et al. 2003). On the other hand, the B-chromosome system in the studied population could be more complicated with a partially parasitic nature of the B-chromosome, as is observed with the chiasma effect (Table 3). It is quite possible that the fitness of B-carrying individuals changes around the year and in different environmental conditions, making them competitively inferior or superior. In such a case, the maintenance of Bs in populations of *A. flavicollis* could be explained by a combination of effects from both heterotic and parasitic models. Such a scenario was already suggested for the Jastrebac population in former Yugoslavia, where balanced frequencies of Bs could result from the elimination of B-carrying animals in spring and early summer in conditions of overabundance and favouring B-bearers in winter survival (B l a g o j e v i ć & V u j o š e v i ć 1995). The problem of population dynamics of Bs in *A. flavicollis* requires further research to resolve the various hypothesis. In general, important information can be gained through further research on meiosis in this species, especially in females, which will

allow one to solve the problem of the presence or absence of an accumulation mechanism in *A. flavicollis*. On the other hand, the studies of the variability levels of progeny of B-bearing parents compared with those from parents without Bs would indirectly indicate the nature of the B-chromosomes in *A. flavicollis*.

A c k n o w l e d g e m e n t s

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