Review Article

Sexual Development

Sex Dev 2011;5:155–166
DOI: 10.1159/000324930

Accepted: January 10, 2011
by G. Scherer
Published online: March 23, 2011

B Chromosomes and Sex in Animals

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Key Words
A chromosomes · B chromosomes · Sex ratio · X chromosome

Abstract
Supernumerary (B) chromosomes are dispensable elements found in many eukaryote genomes in addition to standard (A) chromosomes. In many respects, B chromosomes resemble sex chromosomes, so that a common ancestry for them has frequently been suggested. For instance, B chromosomes in grasshoppers, and other insects, show a pycnotic cycle of condensation-decondensation during meiosis remarkably similar to that of the X chromosome. In some cases, B chromosome size is even very similar to that of the X chromosome. These resemblances have led to suggest the X as the B ancestor in many cases. In addition, sex chromosome origin from B chromosomes has also been suggested. In this article, we review the existing evidence for both evolutionary pathways, as well as sex differences for B frequency at adult and embryo progeny levels, B chromosome effects or B chromosome transmission. In addition, we review cases found in the literature showing sex-ratio distortion associated with B chromosome presence, the most extreme case being the paternal sex ratio (PSR) chromosomes in some Hymenoptera. We finally analyse the possibility of B chromosome regularisation within the host genome and, as a consequence of it, whether B chromosomes can become regular members of the host genome.

Sex Differences in B Chromosome Frequency

Although B chromosomes have frequently been analysed in only one sex, i.e. usually in males because of their cytologically available meiosis, in most B chromosome systems where both sexes have been analysed, they show about similar frequency in males and females. In some exceptional cases, however, B chromosomes are more frequent in a given sex, as detected in either adults or embryo progenies. Sampling of adult males and females in natural populations has shown, for instance, that B chromosomes show similar frequency in both sexes of the grasshoppers *Myrmeleotettix maculatus* [Hewitt and John, 1967] and *Tettigidea lateralis* [Fontana and Vickery, 1973]. We have reviewed this subject in the 2 best sampled populations of the grasshopper *Eyprepocnemis plorans*, i.e. Jete and Salobreña (Granada, Spain). Our analysis has confirmed that B chromosomes showed about the same frequency in males and females in most years sampled, with the only exception of the 1992 sample from Salobreña, where B frequency was significantly higher in males (table 1).

More extremely, B chromosomes were found only in males of the orthopteran *Hemideina crassidens* [Morgan-Richards, 2000]. In the ant *Leptothorax spinosior*, B chromosomes have been found to be frequent in males, but rare in females, and more frequent in testes than somatic tissues [Imai, 1974]. This kind of sex differences have also been reported in vertebrates. For instance, in the characid fish, *Moenkhausia sanctaefilomenae*, all 8 males ana-

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Table 1. Comparison of B frequency between adult males and females of the grasshopper *Eyprepocnemis plorans*

Population	Year	Males			Females			t	df	p
		n	mean Bs	SE	n	mean Bs	SE			
Jete	1977	21	1.048	0.158	14	0.929	0.213	0.45	33	0.657
	1984	44	0.705	0.114	8	1.000	0.250	–1.08	50	0.287
	1985	22	0.955	0.198	12	0.917	0.185	0.14	32	0.890
	1986	169	1.006	0.066	188	1.074	0.059	–0.78	355	0.436
	1990	225	0.862	0.053	132	0.765	0.068	1.13	355	0.258
Salobreña	1984	57	0.807	0.101	8	0.750	0.153	0.31	63	0.757
	1985	35	0.686	0.105	20	0.950	0.180	–1.27	53	0.210
	1990	206	0.869	0.056	82	0.768	0.080	1.04	286	0.301
	1992	237	0.996	0.056	257	0.829	0.047	2.28	492	**0.023**

n = Number of individuals; SE = standard error; t = Student's t test; df = degrees of freedom. Significant test is printed in bold.

Table 2. Sex ratio and sex differences for B frequency in the grasshopper *Myrmeleotettix maculatus*

Population	Bs in		Embryo progeny				χ^2	p	No.		χ^2 (1:1)	p
	mother	father	0B_f	0B_m	1B_f	1B_m			f	m		
East Anglia	1	0	11	12	17	15	0.15	0.698	28	27	0.02	0.893
	0	1	36	44	24	24	0.30	0.583	60	68	0.50	0.480
Tal-y-bont	1	0	4	2	26	21	0.28	0.597	30	23	0.92	0.336
	0	1	33	23	23	30	2.63	0.105	56	53	0.08	0.774
New Forest	0	1	12	12	2	9	3.18	0.075	14	21	1.40	0.237

f = Female; m = male. Association between sex and Bs was tested by contingency χ^2 tests, and sex ratio by a χ^2 test with 1:1 expected frequencies.

lysed carried 1–2 mitotically unstable B chromosomes that were not found in any of the 10 females examined [de Brito Portela-Castro et al., 2001]. The reverse situation has been reported in the characid fish *Astyanax scabripinnis*, where B chromosomes are significantly more frequent in females [Vicente et al., 1996; Néo et al., 2000]. In the frog *Gastrotheca espeletia*, B chromosomes showed about similar frequency in the 4 males and 3 females caught in the wild. However, their offspring, reared in the laboratory after mass crossing, showed significantly higher B frequency in the female sex [Schmid et al., 2002]. Further details on amphibian B chromosomes have been compiled by Schmid et al. [2010].

Sex differences for B frequency can also be investigated in controlled crosses, where B frequency can be tested between male and female progeny. The grasshopper *M. maculatus* is again an example of fair transmission of B chromosomes between sexes. We tested sex ratio (by means of a goodness-of-fit χ^2 test with 1:1 for female:male frequencies) and sex differences in B frequency (by means of a contingency χ^2 test) in the controlled crosses performed by Hewitt [1973] with specimens collected at 3 different populations, and no significant differences appeared (table 2).

We found similar results in a series of mother-offspring analyses in the grasshopper *E. plorans* from the Salobreña population (table 3). In the Jete population, however, the progeny of 55 mother-offspring analyses showed the production of significantly more male than female progeny, and this seemed to occur only among the 1B progeny of 1B mothers (table 3).

However, this male-biased progeny was not associated with significant differences in B frequency between progeny sexes in both the 21 females carrying 1B (mean equal

Table 3. Mother-offspring analysis in the grasshopper *Eyprepocnemis plorans*

Population	Mothers		Offspring					
	Bs	n	Bs	males	females	total	χ^2 (1:1)	p
Salobreña	0	15	0	174	182	356	0.18	0.672
			1	67	71	138	0.12	0.733
			2	1	1	2		
	1	11	0	55	59	114	0.14	0.708
			1	93	73	166	2.41	0.121
			2	23	22	45	0.02	0.881
			3	7	9	16	0.25	0.617
	2	2	0	1	0	1		
			1	7	8	15	0.07	0.796
			2	16	17	33	0.03	0.862
			3	5	5	10	0.00	1.000
			4	2	0	2		
	3	1	1	1	2	3		
			2	7	3	10		
			3	3	7	10		
			Total	462	459	921	0.01	0.921
Jete	0	24	0	289	279	568	0.18	0.675
			1	182	150	332	3.08	0.079
			2	21	23	44	0.09	0.763
			3	3	6	9		
	1	21	0	133	108	241	2.59	0.107
			1	214	168	382	5.54	**0.019**
			2	86	73	159	1.06	0.303
			3	11	8	19	0.47	0.491
			4	1	0	1		
	2	8	0	10	10	20	0.00	1.000
			1	56	71	127	1.77	0.183
			2	48	40	88	0.73	0.394
			3	17	23	40	0.90	0.343
			4	0	1	1		
	3	2	0	4	1	5		
			1	7	10	17	0.53	0.467
			2	12	10	22	0.18	0.670
			3	7	5	12	0.33	0.564
			4	2	2	4		
			Total	1,103	988	2,091	6.32	**0.012**

Significant tests are printed in bold.

to 0.951 in males and 0.947 in females; contingency χ^2 = 0.34, df = 3, p = 0.95, grouping classes with 3 or more Bs) or the 55 females as a whole (mean equal to 0.833 in males and 0.839 in females; contingency χ^2 = 1.20, df = 3, p = 0.75). Since sex determination is performed through spermatids, the most likely explanation for the male-biased sex ratio in the progeny is through an excess of fertilisations performed by X-lacking spermatids and the consequent deficit of fertilisations by X-carrying spermatids. The absence of difference for B frequency between sexes suggests that sex-ratio distortion had nothing to do with B chromosome presence. In fact, sex ratio in the progeny of 1B mothers was about 0.55, irrespectively of the number of Bs in embryos (table 4).

López-León et al. [1996a] observed a significant excess of 1B progeny in 1B × 1B controlled crosses, which they

Table 4. Sex ratio in the progeny of 1B mothers in the grasshopper *Eyprepocnemis plorans*

Number of Bs	Male progeny	Female progeny	Total	Sex ratio
0	133	108	241	0.55
1	214	168	382	0.56
2	86	73	159	0.54
3+	12	8	20	0.60
Total	445	357	802	0.55

explained through the occurrence of negatively assorted gametic fertilisation, after ruling out the possibility of occurrence of other processes such as differential egg resorption by females, segregation distortion favouring the B in one sex, but not in the other, or preferential death of 0B and 2B zygotes during early development. However, in these crosses they did not find sex-ratio distortion, a fact that seems to occur only in gravid females [for possible explanations, see López-León et al., 1996a].

In the migratory locust, *Locusta migratoria*, another acridid orthopteran insect, Pardo et al. [1994] performed 17 controlled crosses; in 8 only the male parent carried B chromosomes, whereas in the remaining 9, only the female parent carried them. In this case, B chromosomes are mitotically unstable, so that each individual is actually a mosaic of cells with a different number of B chromosomes. A summary of their results are shown in table 5. A comparison of B frequency within each cross showed that Bs were significantly more frequent in the male than female progeny in crosses no. 10 and 17 where the B-carrying parent was the father. This difference was due to a female-biased sex ratio in these 2 crosses because many ova from the 0B mother had developed parthenogenetically, thus decreasing B frequency among the female embryo progeny. In cross no. 1, however, the B-carrying parent was the mother and Bs were more frequent among female progeny. This higher B frequency in females is expected from the parthenogenetic development of some of the +B mother's ova, which double the number of B chromosomes during the developmental diploidisation which characterises tychoparthenogenesis in this species [see Pardo et al., 1995]. However, we should also expect a female-biased sex ratio, and this was not observed in this cross (table 5). In 3 other crosses where the B-carrying parent was the mother (nos. 12a, 14 and 15), sex ratio was highly female biased, but no difference in B frequency was found between male and female embryos, perhaps due to the scarce number of males produced. Another interesting parameter to compare among male and female embryo progeny from these crosses is the mitotic instability index (MI) of B chromosomes. As shown in table 5, only one cross showed a significant difference. Despite these differences in some crosses, when we analyse for general tendencies in the 17 crosses, no significant differences were found between male and female embryo progeny for B frequency ($t = 0.37$, $df = 17$, $p = 0.716$), or MI ($t = 0.47$, $df = 17$, $p = 0.641$), but there were significantly more female (1,427) than male (1,127) embryos ($\chi^2 = 35.24$, $df = 1$, $p < 0.001$).

Sex Differences in B Chromosome Effects

We have seen that B chromosomes may sometimes show sex differences in frequency due to a variety of processes such as preferential segregation with the X chromosome during spermatogenesis, in *E. plorans*, or sex-ratio distortion caused by accidental parthenogenesis, in *L. migratoria*. Another source of sex differences can be observed by differential B chromosome effects. For instance, B chromosomes in the mealybug, *Pseudococcus obscurus*, decrease fitness in males but not in females [Nur, 1966a], due to a significant decrease in the number of sperm and increase in developmental time of males [Nur, 1966b]. The formation of abnormal spermatids is frequent in B-carrying grasshoppers [for a recent review, see Teruel et al., 2009], but it has also been reported in butterflies [Pearse and Ehrlich, 1979]. In the grasshopper *M. maculatus*, sperm dysfunction has been reported in B-carrying males [Hewitt et al., 1987]. This species also resembles the mealybug case because its B chromosomes also slow down development, but in both sexes [Hewitt and East, 1978; Harvey and Hewitt, 1979].

No comparable studies exist on the formation of the female gamete because of the higher difficulty for visualisation of oogenesis. However, a reduction in fertility has frequently been reported for B chromosomes in many animal species. As pointed out by Camacho [2005], 'fertility is one of the main fitness components depressed by B presence in most kinds of organisms'. In grasshoppers, egg fertility is a direct measure of female fertility and is frequently lower in B-carrying females. Interestingly, in the grasshopper *E. plorans*, parasitic Bs (e.g. B_{24}, a B variant showing drive) decrease egg fertility significantly [Zurita et al., 1998], but neutralised Bs (e.g. B_2, a non-driving B variant) do not influence it [Camacho et al., 1997], suggesting that suppression of B-drive may also

Table 5. Controlled crosses in *Locusta migratoria* [Pardo et al., 1994]

Cross no.	Father	Mother	Embryo progeny													
			B frequency					sex ratio				mitotic instability index				
			m	f	t	df	p	m	f	χ^2 (1:1)	p	m	f	t	df	p
6	2.29	0	0.88	0.89	0.07	183	0.945	94	91	0.05	0.825	0.112	0.115	0.02	135	0.981
7	1.07	0	0.56	0.53	0.27	150	0.788	71	81	0.66	0.417	0.167	0.168	0.03	75	0.974
8	1.15	0	0.49	0.57	0.62	129	0.537	55	76	3.37	0.067	0.124	0.155	1.00	58	0.319
9	1.93	0	0.87	0.83	0.43	98	0.668	53	47	0.36	0.549	0.109	0.093	0.65	80	0.520
10	1.89	0	0.56	0.33	2.16	179	**0.032**	70	111	9.29	**0.002**	0.064	0.130	2.84	60	**0.006**
11	2.54	0	0.90	0.84	1.50	390	0.135	212	180	2.61	0.106	0.141	0.156	0.96	324	0.336
16	0.91	0	0.38	0.35	0.20	92	0.845	40	54	2.09	0.149	0.100	0.169	1.43	29	0.163
17	3.02	0	1.00	0.52	2.79	58	**0.007**	12	48	21.60	**0.000**	0.079	0.114	0.88	24	0.390
1	0	0.85	0.56	0.74	2.37	190	**0.019**	101	91	0.52	0.470	0.078	0.099	1.19	116	0.238
2	0	1.00	0.47	0.56	0.47	29	0.644	15	16	0.03	0.857	0.145	0.160	0.19	13	0.853
3	0	2.00	0.85	0.95	1.22	241	0.224	121	122	0.00	0.949	0.146	0.117	1.43	180	0.154
4	0	1.00	0.77	0.81	0.58	209	0.564	103	108	0.12	0.731	0.170	0.203	1.60	151	0.112
5	0	2.80	1.64	1.50	0.54	26	0.593	14	14	0.00	1.000	0.066	0.054	0.47	26	0.642
12a	0	0.95	1.17	1.25	0.40	61	0.693	3	60	51.57	**0.000**	0.606	0.329	1.73	42	0.092
12b	0	0.95	0.97	0.72	1.80	68	0.076	34	36	0.06	0.811	0.112	0.098	0.51	51	0.615
13	0	3.11	1.85	2.04	1.33	206	0.186	92	116	2.77	0.096	0.211	0.243	1.67	201	0.097
14	0	2.89	2.16	2.54	1.53	149	0.127	28	123	59.77	**0.000**	0.309	0.254	1.27	145	0.206
15	0	0.86	1.67	1.46	0.50	60	0.618	9	53	31.23	**0.000**	0.270	0.198	1.28	43	0.208

m = Male; f = female; t = Student's t test; df = degrees of freedom. Significant tests are printed in bold.

lead to a decrease of its deleterious effects. But a neutralised B, such as B_2, can still induce deleterious effects in conjunction with harsh environmental conditions, as was observed in B_2-carrying females with shortage of sperm supply and also parasitised by mites genus *Podapolipus* [Muñoz et al., 1998].

Although more studies analysing B chromosome effects separately on males and females are necessary, it could appear that males are more sensitive to B effects than females, but this conclusion could be biased by the easiness of performing analysis in males. For instance, a common effect of B chromosomes is on chiasma frequency [see Camacho, 2005], but this effect has been reported only in males. In *E. plorans*, the intensity of chiasma increase associated with the presence of B chromosomes was higher for parasitic Bs than for neutralised Bs [Camacho et al., 2002]. In the only case where chiasma effects were investigated in both sexes, Cano et al. [1987] did not find significant effects of B chromosomes in this same grasshopper species, but they found that mean chiasma frequency was significantly higher in males than females. In this same species, Henriques-Gil et al. [1989] found an intriguing difference in B chromosome meiotic behaviour in males and females, since B univalents divide reductionally in spermatogenesis, but equationally in oogenesis.

Since both male and female fitness depend on the eggs produced by females, the consequences of B effects are expected to be more severe in females than males. For instance, the fertility reduction derived from the formation of abnormal spermatids rarely would decrease male fertility by more than 10%. Given the huge number of sperm that males are able to produce, this fertility decrease may be insignificant. A reduction of egg fertility, however, could represent significant decrease in female fitness but also in that of the males who fertilised her. It is thus conceivable that B chromosomes which decrease female fitness would have lower evolutionary success than other B variants being more prudent, in which case, we would expect that B chromosomes, in general, would influence less female than male fitness.

Sex Differences in B Chromosome Transmission

Many B chromosomes grant their evolutionary maintenance through a transmission advantage (drive) obtained through a variety of mechanisms, depending on

each case [for review, see Jones, 1991; Camacho, 2005]. B chromosomes usually show drive through one sex only. Examples of drive through males have only been reported in the mealybug *P. obscurus* [Nur, 1962] and the wasps *Nasonia vitripennis* [Beukeboom and Werren, 1993] and *Trichogramma kaykai* [Stouthamer et al., 2001]. In the latter case, however, B chromosomes are present only in males since they transform all fertilised zygotes (destined to be females), carrying them into haploid males by inducing the degeneration of the whole paternal chromosome set accompanying it in the sperm [Werren et al., 1987; Reed and Werren, 1995; Van Vugt et al., 2003].

More frequent is that B chromosome drive takes place in females only, as it has been reported in several grasshopper species [Hewitt, 1973; Lucov and Nur, 1973; Santos et al., 1993; Pardo et al., 1994; Zurita et al., 1998] and rats [Thompson, 1984; Stitou et al., 2004]. In *M. maculatus*, B chromosomes show drive through females but some drag through males [Hewitt, 1973], and in *L. migratoria*, B chromosomes show mitotic instability during embryo development leading to B accumulation in the male germ line, but also show meiotic drive in females [Pardo et al., 1994]. Nevertheless, the extreme case of differential behaviour in transmission between sexes has been reported for germ-line-restricted chromosomes in the finch *Taenopygia guttata* [Pigozzi and Solari, 1998] which, in our opinion, could have derived from conventional B chromosomes. All males carry one of these chromosomes, but all females carry two. In female meiosis, they regularly pair and recombine, and thus all ova carry one. In male meiosis, however, they are completely eliminated from spermatozoa, so that all transmission rests on females [Itoh et al., 2009]. Since the most efficient B chromosome strategy would be maximising transmission while minimising deleterious effects on its host, a conceivable evolutionary destiny of conventional B chromosomes could be this kind of germ-line-restricted chromosomes.

Effects of B Chromosomes on Sex Ratio

In the beetle *Exochomus quadripustulatus*, a correlation between B chromosome frequency and sex ratio was found in a population survey in England [Henderson, 1988]. Among the 14 populations carrying B chromosomes, those displaying higher B frequency showed a higher proportion of females. Due to technical difficulty, females were not analysed for B presence, so it is not possible to test for sex differences in B frequency. Henderson suggested that sex-ratio variation in this species was not causally associated to B chromosome presence but, alternatively, that those factors affecting sex ratio were also responsible for B chromosome differences among populations. As seen above, accidental parthenogenesis in the grasshopper *L. migratoria* can produce both a female-biased sex ratio and an increased B frequency in females because of chromosome doubling during parthenogenetic diploidisation. Population variation for a phenomenon like this in *E. quadripustulatus* could explain the correlation found, bearing in mind that parthenogenesis seems to occur in all main suborders of Coleoptera [Smith, 1971]. However, it is not known whether the English populations analysed show parthenogenesis, and it is also unknown whether it can occur in this species. Recently, Tinsley and Majerus [2007] injected coccinellid male-killing bacteria to several novel hosts, including *E. quadripustulatus*, and this was the only species where no sex-ratio distortion was induced, which appears to suggest that female-biased sex ratios, like those observed by Henderson [1988], are not due to this kind of bacteria, leaving us assuming that it might be caused by other kinds of bacteria-inducing parthenogenesis. Weinert et al. [2007] analysed for the presence of bacteria that are known to cause sex-ratio distortion in ladybirds (*Rickettsia*, *Wolbachia*, *Spiroplasma*, and Flavobacteria), in 21 species of coccinellid beetles. Whereas they found one or more of these bacteria types in 11 of these species, none of them were found in a population of *E. quadripustulatus* from Thetford (UK). However, this population showed a significant male-biased sex ratio (63% of males), whereas neither of those analysed by Henderson showed more than 50% of males. The possibility thus remains that some of Henderson's populations showing up to 70% of females could harbour bacterial symbionts causing sex-ratio distortion, an interesting issue for future research. Another interesting question remaining in this B chromosome system is to estimate B frequency in females. A possibility would be to obtain DNA sequences being specific to the B chromosome and then to analyse molecularly B presence in females.

Another case of parallel variation for sex ratio and B chromosome frequency was reported in the fish *Astyanax scabripinnis*, but here B frequency could be analysed in both sexes. Vicente et al. [1996] found that sex ratio was female biased in the same *A. scabripinnis* populations where B chromosomes were more frequent in females than males. This finding was later confirmed by Néo et al. [2000], who also found that B chromosomes are more frequent in high-altitude populations, perhaps due

to more favourable environmental conditions. To our knowledge, the possible presence of bacterial sex-ratio distorters has not been investigated in this species.

However, the most extreme association between B chromosome presence and sex ratio has been reported in 2 wasps, *N. vitripennis* and *T. kaykai* [for review, see Werren and Stouthamer, 2003]. B chromosomes in these species have been called paternal sex ratio (PSR) chromosomes because they are restricted to males and cause the loss of all paternal A chromosomes accompanying them in sperm, early in development, thus converting diploid fertilised eggs (usually destined to be females) into B-carrying haploid males. The consequence is a male-biased sex ratio and a very high transmission ratio for these B chromosomes. By eliminating the whole host-chromosome set, thus reducing the genetic fitness of B-carrying males to zero, PSR is the most extreme case of genomic parasitism known for any organism [Werren and Stouthamer, 2003]. Recently, Verhulst et al. [2010] have uncovered that the sex-determination system of *Nasonia* depends on the *Nasonia vitripennis transformer (Nvtra)* mRNA. Maternal provision of *Nvtra* mRNA to the oocyte and early zygotic *Nvtra* expression in fertilised eggs lead to female development, whereas maternal imprinting preventing zygotic transcription of *Nvtra* in unfertilised eggs leads to male development. Ascertaining the pathway in which the PSR chromosome disturbs sex-determination converting fertilised eggs into females will provide valuable information on the fine-grain details of the mechanisms underlying this sex-determination system.

B Chromosome Regularisation

B chromosome stabilisation within genomes in diploid organisms requires meiotic regularisation through both sexes, which would imply (i) limiting B chromosome number to two, (ii) always forming a B bivalent, (iii) chiasma formation securing segregation, and desirably, (iv) limiting harmful effects on host fitness. No clear example of B chromosome stabilisation has hitherto been reported for a diploid organism; perhaps because it has never taken place or because it is difficult to get experimental proofs since Bs would be hardly recognisable within a genome where they would have stabilised their behaviour. However, the case of heteropteran psyllids described below [Nokkala et al., 2000, 2003] is nowadays one of the best candidates for B chromosome integration into the A genome.

In haplo-diploid organisms, however, males form gametes through mitosis thus simplifying the problem of meiotic regularisation to only the diploid sex (females). This might explain the apparent stabilisation of B chromosomes in the wasp *Trypoxylon albitarse*, where B chromosome number is limited, in most individuals, to one per haploid genome (1 in males and 2 in females), i.e. the same dosage as the standard (A) chromosomes [Araújo et al., 2001]. The observation of the rapid invasion of this B chromosome in a natural population led Araújo et al. [2002] to postulate that drive is necessary for initial invasion by this B chromosome, but it is directly suppressed by the B tendency to pair and segregate in 2B females. Differently to other B chromosome systems, host-mediated suppression of the B-drive [Nur and Brett, 1985, 1987; Shaw and Hewitt, 1985; Herrera et al., 1996; Camacho et al., 1997] is not necessary in this case because, as argued by Araújo et al. [2002] and Rocha-Sanchez and Pompolo [2004], it is performed by the parasite itself through its tendency to pair during female meiosis.

In diploid organisms, there are several possibilities for B chromosomes to be integrated into the A genome. One is by translocation to an A chromosome. The frequent observation of supernumerary chromosome segments in grasshoppers [Camacho and Cabrero, 1982], which, in many respects, show remarkable similarities with B chromosomes in the same species, suggests the possibility of a common origin for many of them. In fact, some supernumerary segments could actually be integrated B chromosomes. López-León et al. [1991] found a significant positive association between the presence of B chromosomes and supernumerary segments in acridid grasshoppers. The frequent finding of centric fusions between A and B chromosomes [Henriques-Gil et al., 1983; Cabrero et al., 1987] gave support to the possibility that some supernumerary segments arose through this kind of chromosome rearrangement. However, the analysis of a reciprocal translocation between A and B chromosomes in the grasshopper *E. plorans* revealed a substantial decrease in gametic viability associated to the interchange, which highly diminished the chance for a frequency increase, thus showing that interchanges are an unlikely pathway for B chromosome integration into the A genome [Bakkali et al., 2003].

An alternative way for B chromosome integration into the A genome would be finding a counterpart for meiotic segregation. Since all autosomes are by pairs, the only possibility is to segregate preferentially from an unpaired sex chromosome. The first result pointing in this direction was reported in the grasshopper *Phaulacridium vit-*

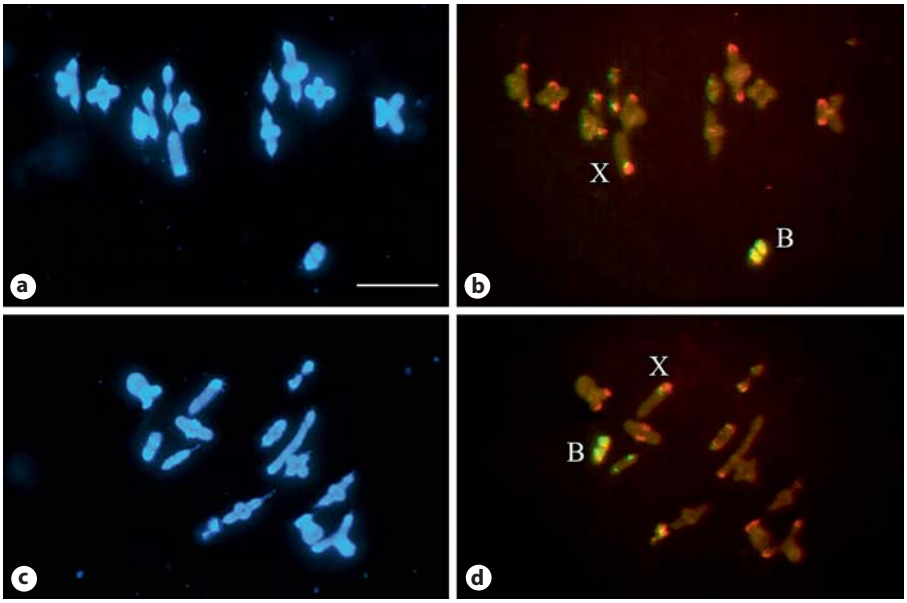

Fig. 1. Metaphase I cells submitted to DAPI (**a**, **c**) and double fluorescence in situ hybridization (**b**, **d**) for a 180-bp satellite DNA (red) and ribosomal DNA (green), showing X-B segregation in the grasshopper *Eyprepocnemis plorans* towards the same pole (**a**, **b**) and to opposite poles (**c**, **d**). Bar = 10 μm.

tatum, where Jackson and Cheung [1967] claimed that X and B chromosomes show preferential migration to opposite poles during first meiotic division, in a natural population from Tasmania. However, John and Freeman [1975] analysed the same population, and other 7 B-carrying populations from Tasmania, and found that X and B segregated randomly in all 56 B-carrying males from the 8 populations. This clearly excluded this Tasmanian population as a case of preferential X-B segregation, bearing also in mind that all other populations analysed from continental Australia showed random X-B segregation [John and Freeman, 1974; Rowe and Westerman, 1974].

In the pygmy grasshopper, *T. lateralis*, X and B chromosomes showed significant preferential migration to the same pole of primary spermatocytes in anaphase I [Fontana and Vickery, 1973, 1975]. The similarity in allocyclic behaviour and the small size difference between X and B led these authors to suggest the origin of this B from the X chromosome. But X and B preferential migration to the same anaphase I pole should produce a B frequency bias in favour of females, since the +X sperm is female determining. However, no sex differences were found for B frequency, presumably because of unequal sample size between sexes. In 1972, only 12 females but 68 males were analysed [Fontana and Vickery, 1973], whereas, in 1973, 40 females and 230 males were sampled. The latter sample has permitted us to estimate that B frequency was about 21% higher in females than males. In addition, Fontana and Vickery [1975] showed that, in the 1973 sample, 206 metaphase II cells carried the X and B chromosomes, whereas 212 did not carry any of them and 318 carried one or the other (these 2 classes were not distinguished). Therefore, 206/736 = 0.28 is the estimated frequency of B chromosomes among the female-determining sperm, whereas, in the absence of B chromosome drive during male meiosis (which is usual in grasshoppers), B frequency in male-determining sperm would be 0.5–0.28 = 0.22. Therefore, B frequency in females should be expected to be 0.28/0.22 = 1.27 times that in males, i.e. 27% higher, a figure not being much different from the observed 21% in this same season.

Random X-B segregation during first meiotic division has been shown in males of the grasshopper *Omocestus burri*, a species where B chromosomes show meiotic drive through females [Santos et al., 1993]. In the grasshopper *E. plorans*, Camacho et al. [1980] found random X-B segregation in 4 populations but preferential segregation in the Otivar population, so that 31% of metaphase II cells from 1B males carried both the X and the B (fig. 1). Sex differences for B frequency, however, could not be tested in this population because females were not analysed. Later, López-León et al. [1996b] reported, in 1B males from samples collected from the Jete population in 1991 and 1992, the occurrence of preferential segregation of X and B chromosomes (31% of metaphase II cells in 1991, and 32% in 1992, carried B but not X chromosomes). This was paralleled to a significantly higher B frequency in males from the 1992 sample, a logical consequence of the

Table 6. X-B segregation in *Rhinocola aceris*

Population	Metaphase II cells with				% to opposite poles	Estimated B freq. females	Estimated B freq. males	Mean Bs in males
	n = 5	n = 6	n = 7	total				
Turku	7	838	7	852	98.4	0.016	0.984	0.949
St. Petersburg	2	170	2	174	97.7	0.023	0.977	1.167
Katowice	0	145	2	147	98.6	0.027	1.000	1.085
Krakow	69	421	69	559	75.3	0.247	0.753	0.303
Tblisi	0	146	3	149	98.0	0.039	1.000	1.000

The estimated B frequency in females was equal to the ratio of n = 7 secondary spermatocytes (those carrying X and B chromosomes) and all female-determining ones (n = 7 plus one half of n = 6 classes). The estimated B frequency in males was calculated as the quotient between the half of n = 6 secondary spermatocytes (those carrying the B but lacking the X) and all male-determining secondary spermatocytes (n = 5 plus one half of n = 6 classes). The mean number of B chromosomes was calculated from table 1 data in Nokkala et al. [2000], averaging available years.

preferential X-B segregation observed in males from 1991. The cases described above might be considered as sporadic attempts for B chromosome stabilisation within the genome through regularising its meiotic behaviour by becoming the sexual counterpart of the X univalent during male meiosis of organisms like grasshoppers bearing an X0/XX sex chromosome determinism. Through an evolutionary pathway like this, a B chromosome could become a Y chromosome. Remarkably, later stages of this hypothetical pathway have been reported in heteropteran psyllid insects. For instance, in *Rhinocola aceris*, Nokkala et al. [2000] found that X and B chromosomes almost always segregated to opposite poles in 4 geographically distant populations and showed preferential segregation in a fifth population (Krakow) (table 6). With this highly regular X-B segregation, we should expect that B chromosomes were almost limited to males. Unfortunately, Nokkala et al. [2000] did not analyse females, but our predictions indicate that these Bs should be very rare in females, except in the Krakow population. This is supported by the remarkable coincidence of our estimates of B frequency in males and those observed in these 5 populations by Nokkala et al. [2000] (see table 6). The observations in *R. aceris* suggest that B chromosomes in this species have been incorporated into an achiasmate segregation mechanism with the X chromosome [Nokkala et al. 2000], which is at different evolutionary statuses among populations, i.e. less advanced in Krakow than in the other populations. These same authors also found a situation in *Psylla foersteri* being very similar to that in the Krakow population of *R. aceris*, i.e. about 74% of X-B segregation. This strongly suggests that B chromosomes in these species might be on the way of turning into Y chromosomes, a process that might have been consolidated in a relative species, *Cacopsylla peregrina*, bearing a true XY sex chromosome system within a group of predominantly X0 species [Nokkala et al., 2003].

Origin of B Chromosomes from Sex Chromosomes, and Vice Versa

B chromosomes show several similarities with sex chromosomes such as pycnotic cycle and univalency during meiosis or accumulation of repetitive DNA families [see Camacho et al., 2000]. This suggests a certain parallelism between these two kinds of chromosomes. Of course, sex chromosomes may be the source of B chromosome origin, as suggested for the Y chromosome in the fly *Glossina* [Southern and Pell, 1973] and the W chromosome in the frog *Leiopelma hochstetteri* [Green, 1988]. In *Glossina*, similitude in meiotic behaviour seemed to point to B origin from the Y [Southern and Pell, 1973], but in situ hybridization analysis with repetitive DNAs showed a complex picture [Amos and Dover, 1981]. In *L. hochstetteri*, however, B derivation from the W chromosome was inferred after DNA sequence comparison [Sharbel et al., 1998], which offers much higher confidence in that conclusion.

In the grasshopper *E. plorans*, López-León et al. [1994] suggested that B chromosomes had derived from the X chromosome on the basis of chromosome distribution of a tandem repeat DNA and ribosomal DNA (rDNA) in respect to the centromere, but recent analysis of ITS DNA sequence has ruled out this possibility [Teruel et al., in preparation]. Likewise, in 2 South American grasshopper

species, B chromosomes being very similar, in size and meiotic behaviour, to the X chromosome have been shown to be most likely of autosomal origin, on the basis of chromosome distribution of 45S and 5S rDNA [Loreto et al., 2008]. This calls for caution in inferring B chromosome origin from chromosome morphology, size or meiotic behaviour. The most appropriate tool is DNA-sequence comparison between A and B chromosomes. For instance, in the migratory locust, the presence of a cluster of histone genes in the B chromosome has suggested that they derived from the eighth autosome, in order of decreasing size, which is the only A chromosome carrying a histone gene cluster [Teruel et al., 2010]. DNA-sequence comparison of H3 and H4 histone gene copies obtained from the A and B chromosomes, separately, led these authors to suggest that these B chromosomes originated about 750,000 years ago.

It is thus possible that some B chromosomes would have derived from sex chromosomes, but the only clearly shown case is that reported by Sharbel et al. [1998] in the frog *L. hochstetteri* (see above). But the other way around is also conceivable, i.e. that some sex chromosomes have descended from B chromosomes. The clearest cases are those described above in heteropteran psyllids [see Nokkala et al., 2000, 2003], since intermediate and final stages of B chromosome conversion into a Y chromosome are observed in a single species, *R. aceris*, and similar intermediate and final stages are observed in relative species like *P. foersteri* and *C. peregrina*, respectively (see above). In addition, it has been suggested that the Y chromosome in *Drosophila* derived from a B chromosome [Hackstein et al., 1996; Carvalho, 2002]. A recent comparison of DNA sequence among the Y chromosomes in 12 *Drosophila* species with sequenced genomes [Koerich et al., 2008; Carvalho et al., 2009] has shown interesting insights. In *Drosophila melanogaster*, the Y chromosome does not share any single-copy genes with the X, thus suggesting that the Y did not derive from the X, which is the canonical hypothesis. The *D. melanogaster* Y chromosome actually contains few (12–20) single-copy protein-coding genes, all of them derived from duplication of autosomal genes, and have functions related with male fertility [for review, see Carvalho et al., 2009]. In addition, it has been shown that gene content in the *Drosophila* Y chromosome is much younger than that in the remaining chromosomes and that the Y has suffered 10.9 times more gene gains than losses [Koerich et al., 2008]. On the basis of these observations, Carvalho et al. [2009] suggested a non-canonical origin of Y chromosomes in *Drosophila* by which they would have derived from B chromosomes that first acquired the capacity to pair with the X chromosome and later gained the male fertility genes from the autosomes, a hypothesis being highly consistent with observations in heteropterans [see Nokkala et al., 2000, 2003]. As shown in the migratory locust [Teruel et al., 2010], microdissection can be a useful tool to compare DNA sequences among chromosomes within genomes. The Y chromosome richness in repetitive DNA precludes the final assembly of its complete DNA sequence in genome projects, but these same repetitive DNAs might be useful to compare with similar sequences in the other chromosomes and thus could be a gold mine to rebuild the history of certain chromosomes, including the Y, in many species.

Acknowledgements

This study was supported by grants from the Spanish Ministerio de Ciencia y Tecnología (CGL2009-11917) and Junta de Andalucía (CVI-6649), and was partially performed by FEDER funds.

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