

# Integration of a B chromosome into the A genome of a wasp, revisited

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A previous study showed that in the haplodiploid solitary wasp *Trypoxylon albitarse*, most individuals carry one B chromosome per haploid genome, the same dosage as the standard (A) chromosomes, indicating a possible regularization of B-chromosome meiotic behaviour and its integration into the A genome. In a new sampling, we have analysed 15 populations (including 9 out of the 10 previously analysed) to test the evolution of this integration process. The new results provide a direct report of the invasion process in the Porto Firme population, where B frequency has dramatically increased in only four generations. In the populations from the Viçosa region, however, B frequency has remained stable, although the principal B type, the metacentric one, has increased in frequency at the expense of the acrocentric one in several populations. The implications of these new results on the hypothesis of the integration of these B chromosomes, as regular members of the A genome, are discussed.

**Keywords:** B-chromosome frequency; population dynamics; *Trypoxylon albitarse*; drive; meiotic behaviour; integration

## 1. INTRODUCTION

B chromosomes are genome parasites maintained in natural populations of many eukaryotic organisms at the expense of a variety of accumulation mechanisms (i.e. drive), in spite of their harmful effects on the host genome (Camacho *et al.* 2000). In nature, cases of coevolution towards mutualism have been reported, the most popular being the mitochondria in the eukaryotic cell. Explaining how this coevolutionary change takes place in natural populations is one of the most challenging issues of evolutionary biology. In a previous paper, we supplied evidence that B chromosomes in the solitary haplodiploid wasp *Trypoxylon albitarse* are in the process of becoming regular members of the chromosome complement, because most individuals carried a single B per haploid genome, the same dosage as the standard (A) chromosomes (Araújo *et al.* 2001). We thus postulated that these B chromosomes need drive (i.e. a segregational mechanism favouring the increase of B frequency in the germ line) when they first invade a population, but it is then lost when the B number is regulated to one per haploid genome, most probably because of a high tendency of two Bs to pair in female meiosis. Under this scenario, B invasion and stabilization would be expected to be rapid, and both should lead to the presence of one B per haploid A genome in most individuals.

Our present work tests these expectations in a new sampling including nine populations previously analysed, and six new populations of the wasp *T. albitarse* (Hymenoptera: Sphecidae: Larrinae). The results met all expectations of a rapid invasion and integration process,

and indicate that the metacentric B is fitter than the acrocentric one.

## 2. MATERIAL AND METHODS

A total of 637 individuals (284 females and 353 males) from 533 nests of *T. albitarse* were collected from 15 natural populations in five municipalities (Viçosa, Porto Firme, Cajuri, Coimbra and Piranga) in the forest zone of Minas Gerais state (Zona da mata mineira, Brazil), from January 1998 to December 2000 (table 1). In Viçosa and Porto Firme, we resampled 9 out of the 10 populations analysed previously (Araújo *et al.* 2001).

The methods for cytological and statistical analyses were essentially the same as in our previous report (Araújo *et al.* 2001). The five variables employed for analysis were: (i) the number of B chromosomes per haploid A genome ( $B_{hg}$ ); (ii) and (iii) the number of metacentric or acrocentric B chromosomes per haploid A genome ( $Met_{hg}$  and  $Acro_{hg}$ , respectively); (iv) the stabilization index (SI) of B chromosomes ( $B_{SI}$ ), i.e. the proportion of individuals carrying one B per A haploid genome (Araújo *et al.* 2001); and (v) the SI of the metacentric B chromosome ( $Met_{SI}$ ). In the Porto Firme population, some individuals showed intra-individual variation in B number. For statistical analysis, we used the most frequent B number in these mosaic individuals.

## 3. RESULTS AND DISCUSSION

We first analysed temporal variation in the populations sampled twice in the Porto Firme and Viçosa municipalities. The Porto Firme population (Nova Ilha) has shown a spectacular increase in B frequency in only two years, i.e. four generations (table 2). This increase has been mainly achieved by the metacentric B, which was the only B variant present in the first sampling and the most

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Table 1. Number of nests collected and individuals analysed cytologically in the new sampling. (The first nine populations were also sampled in Araújo *et al.* (2001).)

population	district	geographical coordinates	nests	specimens analysed	
				females	males
Nova Ilha (Nil)	Porto Firme	20°43'21" S, 43°05'33" W	36	32	30
Amoras (Amo)	Viçosa	20°42'46" S, 42°54'08" W	43	20	18
Campus (Cam)	Viçosa	20°45'54" S, 42°51'31" W	60	22	28
Vila Cristal (Cri)	Viçosa	20°46'08" S, 42°50'17" W	53	30	29
Palmital (Pal)	Viçosa	20°49'20.9" S, 42°50'58.3" W	37	35	39
Paraíso (Par)	Viçosa	20°50'05" S, 42°50'58" W	18	8	9
Marrecos (Mar)	Viçosa	20°50'17" S, 42°51'51" W	38	8	21
Silvestre (Sil)	Viçosa	20°43'34" S, 42°52'45" W	21	10	8
Vila Chaves (Cha)	Viçosa	20°45'05" S, 42°50'38" W	14	12	20
Barrinha (Bar)	Viçosa	20°45'11.5" S, 42°52'49.5" W	58	22	36
Fundao (Fun)	Viçosa	20°44'55.6" S, 42°49'28.7" W	36	15	22
Cajuri (Caj)	Cajuri	20°47'17.4" S, 42°47'39.7" W	67	29	53
Vila Paraguai (Vpa)	Cajuri	20°45'38.3" S, 42°44'58.3" W	20	10	16
Coimbra (Coi)	Coimbra	20°51'21.9" S, 42°48'15.2" W	10	10	14
Piranga (Pir)	Piranga	20°40'18.7" S, 43°18'28.3" W	22	21	10
total			533	284	353

Table 2. Comparison of five population parameters between the two sampling periods in the Nova Ilha population (Porto Firme region) and the eight populations from the Viçosa region, by means of the Mann–Whitney test. (*n*, number of nests analysed; *P*<sub>b</sub>, probability corrected for the sequential Bonferroni method.)

	Porto Firme					Viçosa				
	1997	1999	<i>U</i>	<i>P</i>	<i>P</i> <sub>b</sub>	1996–1997	1998–2000	<i>U</i>	<i>P</i>	<i>P</i> <sub>b</sub>
	mean ( <i>n</i> = 10)	mean ( <i>n</i> = 36)				mean ( <i>n</i> = 146)	mean ( <i>n</i> = 173)			
<i>B</i> <sub>hg</sub>	0.133	0.962	24.5	0.000 035	0.000 175	0.993	0.937	11 030.5	0.051 456	
Met <sub>hg</sub>	0.133	0.883	38.5	0.000 165	0.000 660	0.810	0.908	10 513.0	0.009 933	0.029 7990
Acro <sub>hg</sub>	0	0.079	155.0	0.505 557		0.183	0.029	7 295.5	< 0.000 001	< 0.000 005
<i>B</i> <sub>SI</sub>	0.133	0.529	91.0	0.017 786	0.053 358	0.904	0.693	9 015.5	0.000 011	0.000 0440
Met <sub>SI</sub>	0.133	0.436	113.0	0.074 386		0.633	0.673	11 589.0	0.205 089	

frequent one in the second. The acrocentric B appeared in the second sampling at a very low frequency (figure 1a). This indicates that the acrocentric B is most probably a by-product derived from the metacentric B through deletion and/or inversion (Araújo *et al.* 2000). The high mutability of B chromosomes generating new variants is characteristic of some B-chromosome systems, e.g. the grasshopper *Eyprepocnemis plorans* (López-León *et al.* 1993), and is one of the key properties granting a long life for B-chromosome polymorphisms (Camacho *et al.* 1997).

This dramatic increase in B frequency provides direct evidence of an invasion episode by the metacentric B, which has passed from a mean frequency of 0.133 in the first sampling to 0.883 in the second. The only precedent of a direct report of B invasion in a natural population was shown in *E. plorans* (Zurita *et al.* 1998). In parallel to the increase in B frequency, *B*<sub>SI</sub> and Met<sub>SI</sub> have increased up to values close to 0.5 (figure 1b,c), although they are still lower than those observed in Viçosa (see table 2). The occurrence of some mosaic individuals, showing cells with different numbers of B chromosomes, as well as the presence of some males with 0B or 2B and some females with

1B, 3B or 4B, might explain the lower SI in Porto Firme. In the Viçosa region, the mean frequency of mosaics was 2.5% among males and 7% among females. In Porto Firme, however, we found 3.3% of mosaics in males and 31.25% in females. It is clear that B instability is lower in males (haploid) than females (diploid), and that instability in females is much higher in Porto Firme than Viçosa. It is interesting to note that the Porto Firme population was analysed during a period in which the B frequency has remarkably increased (indicating a recent invasion), but populations from the Viçosa region have been analysed once the B frequency was high (indicating an older invasion). It is thus probable that B chromosomes in Viçosa populations have had more time for mitotic regularization in the diploid sex. In Porto Firme, the B chromosome has had to face new genetic backgrounds, some of which may not facilitate its regular transmission. These signs of instability are commonly found in B chromosomes to a higher or lower degree (for a review, see Jones & Rees (1982)).

In Viçosa, the eight re-analysed populations, as a whole, showed stability for *B*<sub>hg</sub> (figure 1a) and Met<sub>SI</sub> (figure 1b),

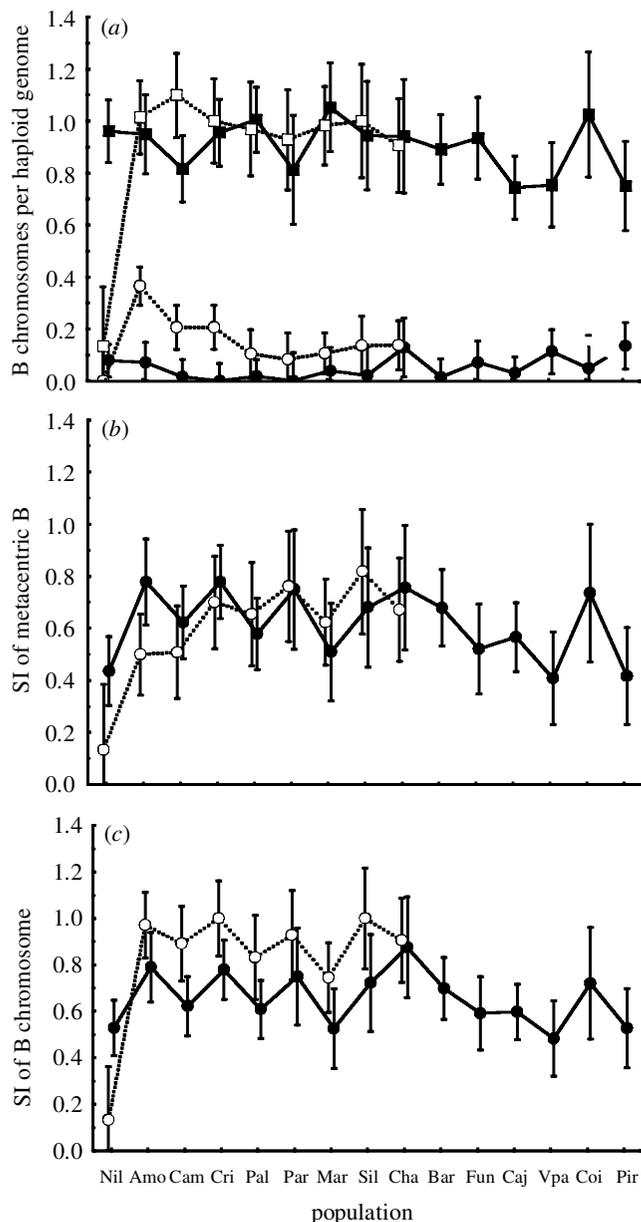


Figure 1. B-chromosome evolution in 15 Brazilian populations of the wasp *Trypoxylon albitarse*. (a) Total number of B chromosomes ( $B_{hg}$ ; squares) and number of acrocentric chromosomes ( $Acro_{hg}$ ; circles) per haploid A genome. (b) SI for the metacentric B chromosome ( $Met_{SI}$ ). (c) SI for all kinds of B chromosomes ( $B_{SI}$ ). Populations analysed by Araújo *et al.* (2001) are represented by open squares or circles and are joined by a dotted line. See population codes in table 1.

but there were significant tendencies for  $Met_{hg}$  to increase and  $Acro_{hg}$  to decrease (figure 1a). These changes in the frequency of these two B chromosomes have been paralleled by a decrease in  $B_{SI}$  (table 2), and were particularly apparent in Amoras, Campus and Vila Cristal populations (figure 1c). These results indicate an overall stability in B frequency in this region, although it is only apparent because a replacement of the acrocentric B by the metacentric B seems to be taking place. The substitution of one B variant for another has previously been reported in the grasshopper *E. plorans* and was based on the existence of drive for the replacing variant (Zurita *et al.* 1998). In

*T. albitarse*, we have no data on B-chromosome transmission, but the frequency data indicate that transmission efficiency of the metacentric B is higher than that of the acrocentric B. Bearing in mind that the metacentric is the most frequent B in all populations hitherto analysed and that its invasion ability seems to be very high (as was observed in Porto Firme), it is reasonable to argue that the metacentric B is fitter than the acrocentric one.

The six new populations sampled showed B frequency and stability parameters similar to those observed in the Viçosa region, indicating that they are at a similar evolutionary stage, with most individuals carrying one B per haploid genome and high SI values (see figure 1), which is logical given their proximity to Viçosa.

As a whole, our present results give strong support to our hypothesis on the integration of B chromosomes into the A genome of *T. albitarse* by the joint action of drive, which is necessary for the initial invasion, and regularization of B meiotic behaviour in the diploid sex, presumably because of a high tendency of Bs to pair. We cannot rule out, however, that bearing one B per haploid genome might be beneficial for the host genome, in which case, invasion would also be rapid. An invaded population would rapidly tend to show most of its individuals harbouring one B per haploid genome, provided that drive (or selection) is strong and meiotic pairing of the two Bs, in 2B females, tends to suppress drive.

The possibility remains that the decrease in the frequency of the acrocentric B and the consequent overall decrease in SI is the result of the 'arms race' between the A and B chromosomes (Camacho *et al.* 1997). In other B-chromosome systems, the usual outcome of the arms race is the suppression of B drive (Nur & Brett 1985, 1987, 1988; Shaw & Hewitt 1990; Herrera *et al.* 1996; Camacho *et al.* 1997). In *T. albitarse*, if our hypothesis is correct, drive is suppressed by the regularization of B meiotic pairing. For this reason, host-mediated suppression of drive is not necessary because it is achieved by the parasite itself. The existence of a low degree of B mitotic instability and a certain rate of mutation of the metacentric into the acrocentric B, which seems to be less fit, are two additional obstacles that the metacentric B needs to pass to become a regular member of the A-chromosome complement.

We thank M. J. P. Araújo for his assistance in the collection of specimens and C. C. Silva for her laboratory assistance. This study was in part supported by grants from Fundação de Amparo à Pesquisa de Minas Gerais, Coordenação de Aperfeiçoamento de Pessoal de nível Superior and Conselho Nacional de Pesquisa (S.M.S.R.A. and S.G.P.), and Ministerio de Ciencia y Tecnología (BOS2000-1521) and Junta de Andalucía (CVI-165) (F.P. and J.P.M.C.).

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