THE ROYAL SOCIETY

doi 10.1098/rspb.2001.1613

Integration of a B chromosome into the A genome of a wasp

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B chromosomes are genome symbionts, the presence of which in many eukaryote species is explained, in most cases, by their violation of Mendelian rules, usually based on meiotic or mitotic instability, leading to their accumulation in the germ line (drive). However, B chromosome integration into the genome as a regular member of the chromosome set should imply the loss of drive. A possible way of bypassing this difficulty is to regularize meiosis when the B chromosome is frequent in the population, in order to yield gametes with one B chromosome. In diploid organisms, this task needs to be achieved in the two sexes, but in haplodiploids the problem simplifies to only the diploid sex. We have found, to the authors' knowledge, the first evidence of a B chromosome that is regularizing its meiotic behaviour and limiting its number to one B chromosome per haploid genome, the same dosage as the standard (A) chromosomes, in the solitary wasp *Trypoxylon albitarse*. It suggests a possible mechanism for B chromosome integration as a regular member of the chromosome complement.

Keywords: B chromosomes; genomic parasitism; *Trypoxylon albitarse*

1. INTRODUCTION

Some individuals in many eukaryote populations harbour dispensable extra chromosomes, the so-called B chromosomes (Jones & Rees 1982). These may be considered as genomic symbionts, which, in the majority of cases studied, exhibit parasitic behaviour to the detriment of their host genome (Jones & Rees 1982; Beukeboom 1994; Camacho *et al.* 2000). As a rule, B chromosomes lack the regular meiotic behaviour that guarantees normal chromosome segregation and Mendelian inheritance for the autosomes (two homologous chromosomes segregating to different gametes). This irregularity may constitute the basis for B chromosome accumulation in the germ line through a variety of drive mechanisms that enable B chromosome maintenance in natural populations (Jones 1991). However, B chromosome drive also impedes B chromosome stabilization as a normal member of the A chromosome set. Theoretical solutions to this trade-off could be either B chromosome elimination (Camacho *et al.* 1997) or B chromosome integration into the A genome with the subsequent increase in chromosome number.

The possibility of B chromosome integration into the host genome was pointed out long ago. One view suggested that mode of integration involves coevolutionary changes in the B chromosome and the A genome leading to the attenuation of B chromosome effects to a null or even beneficial state (Kimura & Kayano 1961; Hewitt 1973). However, theoretical and empirical research has indicated that the arms race between the A and B chromosomes could also lead to an endless cycle of parasitic B chromosome neutralization by the host genome, followed by B chromosome mutations which re-establish transmission drive to begin a new cycle (Camacho *et al.* 1997). A second mechanism of B chromosome integration has been suggested by the observation of spontaneous fusions between A and B chromosomes in the grasshopper *Eyprepocnemis plorans* (Henriques-Gil *et al.* 1983; Cabrero *et al.* 1987) and maize (Maguire 1995). Although this possibility cannot be ruled out, the fact that neither of these chromosome fusions has invaded natural populations suggests the existence of major constraints on their frequency increase in natural populations. Alternatively, a more direct mode of B chromosome integration would be to imitate A chromosomes by acquiring regular meiosis, i.e. consistent pairing during prophase in both sexes (in diploid organisms) and segregating one B chromosome to each anaphase-I pole and, thus, to each gamete. The need for this double achievement (in both male and female meiosis) is probably a major barrier to this path of B chromosome integration in diploid organisms. In fact, most B chromosomes in diploid organisms only accumulate in one sex, which is evidence that male and female meiosis may not be identical from a B chromosome's perspective. However, in haplodiploids, meiosis only occurs in one sex (the diploid one) and, hence, this may provide a more suitable context in which B chromosome integration may evolve.

In the present work, we report evidence of a B chromosome, the number of which is being limited to one B chromosome per haploid genome, the same dosage as the standard (A) chromosomes, in the wasp *Trypoxylon albitarse*. It suggests a possible mechanism for B chromosome integration as a regular member of the chromosome complement.

2. MATERIAL AND METHODS

Trypoxylon albitarse (Hymenoptera: Sphecidae: Larrinae) is a *ca.* 2.5-cm-sized, solitary, haplodiploid wasp inhabiting mud nests and showing a wide distribution in South America. The chromosome number of these wasps is known in only 12 out of 355 *Trypoxylon* species. Among these 12 species, chromosome

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Received 16 October 2000 *Accepted* 9 February 2001

Table 1. *Populations sampled, location and number of nests and larvae analysed*

population	district	geographical coordinates	nests	larvae	
				female	male
Amoras	Viçosa	20°42′ 46″ S, 42°54′ 08″ W	26	30	26
Campus	Viçosa	20°45′ 54″ S, 42°51′ 31″ W	20	22	24
Centro	Viçosa	20°45′ 05″ S, 42°52′ 50″ W	6	1	7
Marrecos	Viçosa	20°50′ 17″ S, 42°51′ 51″ W	22	34	36
Nova Ilha	Porto Firme	20°43′ 21″ S, 43°05′ 33″ W	10	9	12
Palmital	Viçosa	20°49′ 20.9″ S, 42°50′ 58.3″ W	18	28	27
Paraiso	Viçosa	20°50′ 05″ S, 42°50′ 58″ W	12	7	14
Silvestre	Viçosa	20°43′ 34″ S, 42°52′ 45″ W	11	7	11
Vila Chaves	Viçosa	20°45′ 05″ S, 42°50′ 38″ W	15	20	11
Vila Cristal	Viçosa	20°46′ 08″ S, 42°50′ 17″ W	19	21	19

number varies from $2n = 18$ to $2n = 32$ (Hoshiba & Imai 1993). However, very little is known about the behavioural ecology of this species. Each nest is occupied by a single female and a single guarding male, whose paternity proportion of the nest brood is unknown. Females provide food (spiders) for each nest cell before laying an egg in it. The offspring in a single nest exist at various developmental stages since eggs are laid sequentially depending on when the cells contain enough food for future larvae.

Between 1996 and 1997, we sampled a total of 366 *T. albitarse* larvae from 159 nests collected at ten natural populations in the state of Minas Gerais, Brazil (table 1). We collected all available larvae in each nest but, due to sequential development, only a fraction of offspring per nest were at the appropriate stage for cytological analysis. We analysed one male and one female in most nests, but in a few nests we analysed only one individual (of either sex) and in a few others we analysed several individuals of the same sex (up to four). The cytological analyses (mainly C-banding and fluorochrome staining) were performed on the cerebral ganglia of larvae at the post-defecating stage, according to the techniques described in Araújo *et al.* (2000).

Statistical analysis of the data was performed by giving the same weight to each nest, independent of the number of individuals analysed, in order to avoid pseudo-replication due to the non-independence of a same nest's individuals. For this purpose, a mean per nest was obtained for any variable in those nests where several individuals had been analysed. All statistical comparisons were performed by means of non-parametric tests, because all variables did not satisfy normality.

In order to compare the B-chromosome numbers in males and females, we calculated the numbers of B chromosomes per haploid A genome (B_{hg}) and compared them between sexes by the Mann–Whitney *U*-test in each population separately. Kruskal–Wallis ANOVA was used for testing differences between populations.

Meiotic stabilization is a possible process by which a B chromosome might preserve its existence in the host genome. A B chromosome that acquires meiotic behaviour similar to that of the A chromosomes, i.e. pairing at prophase and segregating to opposite poles at anaphase, would secure its presence in all gametes. Such an achievement in a haplodiploid organism such as *T. albitarse* would inevitably lead to all females carrying two B chromosomes and all males carrying one B chromosome, i.e. one B chromosome per haploid A genome. In order to quantify the stabilization process of the B chromosome, we calculated a stabilization index (SI), which is defined as the proportion of individuals carrying one B chromosome per haploid A genome. For a diploid organism, this will coincide with the proportion of individuals carrying two B chromosomes. Differences between populations were tested by Kruskal–Wallis ANOVA and differences between sexes by the Mann–Whitney *U*-test. Temporal variation in the SI of each population was tested by Kruskal–Wallis ANOVA.

We compared a matrix of between-population geographical distances with one of between-population SI differences by means of a Mantel test in order to measure the degree of relationship between both matrices and, thus, investigate possible SI spatial patterns. The significance of the test was obtained by permutation.

3. RESULTS

In addition to the standard A genome consisting of 32 chromosomes in females and 16 chromosomes in males, two types of B chromosomes were found. Both types of B chromosome were completely heterochromatic, with the predominant type being metacentric and the other acrocentric. The acrocentric B chromosome could be a variant derived by deletion or pericentric inversion, since both B chromosome variants showed very similar responses to the C-banding and fluorochrome techniques (Araújo *et al.* 2000). On this basis, and from the absence of male larvae carrying the two B chromosome types in 15 nests where the mother carried both B chromosome types (as deduced from her progeny), we decided to consider both B chromosome types as a whole for subsequent analyses.

Most females in the populations from the Viçosa region carried two B chromosomes and most males carried a single one (figure 1). This observation suggests that a process of B chromosome stabilization is taking place in the genome of *T. albitarse*. Such a process might have been completed in three populations (Vila Cristal, Silvestre and Centro; table 1), where all females carried two B chromosomes and all males carried one. In Nova Ilha, the single population analysed from the Porto Firme region, B chromosomes were found in only three males from three different nests (27.3%). This suggests that B chromosomes might have invaded this locality recently.

The numbers of B chromosomes per haploid A genome (B_{hg}) were compared between sexes and populations. No

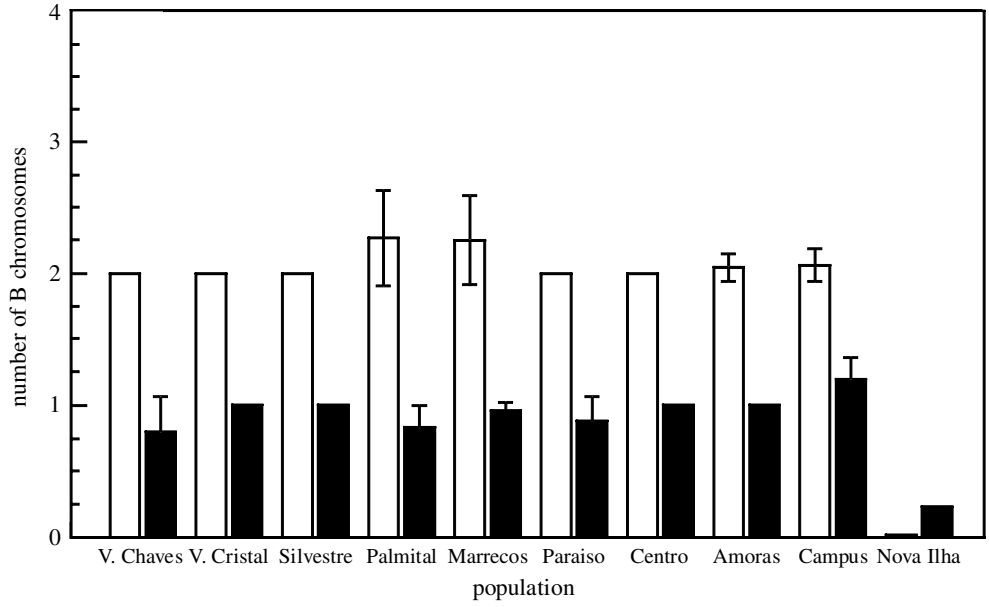

Figure 1. B chromosomes in ten Brazilian populations of the wasp *T. albitarse*. Open bars correspond to females and solid bars to males.

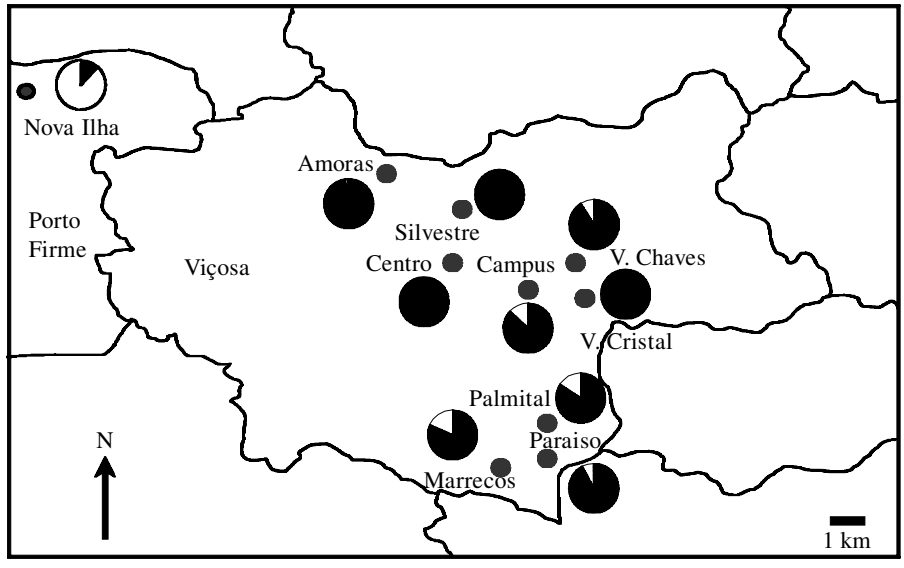

Figure 2. Geographical variation in the SI.

significant differences were found between the sexes in any of the ten populations (results not shown), but the differences between populations were significant (Kruskal–Wallis ANOVA, $H = 100.6$, d.f. $= 9$ and $p < 0.0001$). The same result was obtained for the metacentric ($H = 45.6$, d.f. $= 9$ and $p < 0.0001$) and the acrocentric ($H = 24.0$, d.f. $= 9$ and $p = 0.0043$) B chromosomes separately.

We calculated the SI per nest, sex and population (figure 2). The SI differed significantly between populations (range 0.118–1.00) ($H = 98.39$, d.f. $= 9$ and $p < 0.0001$) but not between sexes in any population (results not shown). The SI for the nine populations from the Viçosa region also differed significantly between populations ($H = 24.08$, d.f. $= 8$ and $p = 0.0022$), although the range was much shorter (0.816–1.00). A comparative analysis of the SI between the four reproductive seasons studied showed no significant differences in most populations, with the exception of the Campus population (one of the most intensively sampled), which showed a significant tendency towards stabilization during this two-year period ($H = 12.31$, d.f. $= 3$, $n = 33$ nests and $p = 0.0064$). The B chromosome was unstable in the first season (as deduced from the presence of several males with two B chromosomes), but stable in the other three successive seasons. The B chromosome was completely stabilized or very close to being completely stabilized in populations from the Viçosa region, where the B chromosome frequency was high (figure 2). In contrast, the population with a low B chromosome frequency (Nova Ilha) was rather far from B chromosome stabilization. A Mantel test comparing a

matrix of geographical distances between populations with one of population differences in the SI showed a very good fit between both matrices ($Z = 0.881$ and $p = 0.022$). This is consistent with B chromosome invasion from a centre of origin that was probably focused on the Centro and Silvestre populations.

4. DISCUSSION

Our results are completely novel and illustrate a simple mechanism for B chromosomes integrating themselves into the A genome, i.e. their stabilization to the number of one B chromosome per haploid A genome (B_{hg}). Several populations (Vila Chaves, Silvestre and Centro) seemed to be at or near B chromosome stabilization, since all nests analysed contained individuals with a B_{hg}-value of unity. The remaining populations from the Viçosa region showed a B_{hg}-value close to unity, although some variation was explained by the presence of some males with no or two B chromosomes and some females with one, three or four B chromosomes. These signs of instability are characteristic of all known B chromosomes, although at a much higher degree (Jones & Rees 1982). For comparison, we calculated the SI for B chromosomes in several species. The SI was 0.036 in *Crepis capillaris* (Parker *et al.* 1991), 0.179 in *Allium schoenoprasum* (Holmes & Bougourd 1989), 0.020–0.250 in maize (Rosato *et al.* 1998), 0.097–0.130 in the mouse *Apodemus flavicollis* (Vujošević & Blagojević 1995), 0.034–0.452 in the grasshopper *Myrmeleotettix maculatus* (Hewitt & John 1970) and 0.400 in the grasshopper *E. plorans* (Zurita *et al.* 1998). In sharp contrast, the B chromosome of *T. albitarse* reached SI values of 0.816–1.00 in the Viçosa region.

It has been shown in the grasshopper *E. plorans* that B chromosomes need drive in order to invade populations and establish a polymorphism (Camacho *et al.* 1997). It is likely that, in *T. albitarse*, B chromosomes show meiotic drive in females at the initial phases of B chromosome invasion but that, when the B chromosome reaches high frequency, the drive be masked by regular B chromosome pairing and segregation during female meiosis. An extremely high tendency for regular B chromosome pairing in females with two B chromosomes, thus forming ova with one B chromosome, could therefore be the key to B chromosome stabilization in *T. albitarse*. It is clear that the possibility for this kind of stabilization is easier in haplodiploid organisms because pairing and regular segregation only needs to be achieved in the diploid sex (see § 1), although regular mitotic behaviour of the B chromosome during spermatogenesis is also needed. It has been suggested that the Y chromosome in *Drosophila* was possibly derived from a B chromosome (Hackstein *et al.* 1996). In this case, meiotic regularization would remarkably only also need to be achieved in one sex.

Pairing of B chromosomes has been suggested as a mechanism for avoiding meiotic loss (Carlson & Roseman 1992). These authors proposed that the typical nondisjunction of some B chromosomes at meiosis increases the number of individuals with two B chromosomes, thus minimizing B chromosome meiotic loss by increasing B chromosome bivalent frequency. For the same reason, pairing between the two B chromosomes avoids their meiotic accumulation.

B chromosomes are rare in Hymenoptera, the scarce cases that have been reported occurring in only five ant species, i.e. *Leptothorax spinosior* (Imai 1974), *Aphaenogaster rudis* (Crozier 1975), *Podomyrma adelaide* (Imai *et al.* 1977), *Pseudolacius* sp. 2. and *Prenolepis jerdoni* (Imai *et al.* 1984), two wasp species, i.e. *Nasonia vitripennis* (Werren 1991) and *T. albitarse* (Araújo *et al.* 2000; this paper) and a bee, i.e. *Partamona cupira* (Costa *et al.* 1992).

The 'paternal sex ratio' (PSR) B chromosome in the wasp *N. vitripennis* avoids meiotic loss by only being transmitted by the haploid sex (males) and, thus, bypassing meiosis in the diploid sex (females) (Werren 1991). The PSR is transmitted through male gametes that fertilize ova and produce females. However, the PSR causes condensation and loss of the paternal chromosomes accompanying it, thereby transforming the diploid zygote into a haploid B chromosome-carrying male (Werren *et al.* 1987).

Several evolutionary strategies could be followed by B chromosomes in order to persist in populations. The most widespread is to have accumulation mechanisms (e.g. chromosome meiotic drive or mitotic instability), but other strategies are also possible, including beneficial fitness effects to the host. This is the case for heterotic B chromosomes, which are represented by the B chromosome of the chive *A. schoepranoprasum*, which improves survival from seed to seedling (Holmes & Bougourd 1989; Bougourd *et al.* 1995) or the B chromosome in the fungus *Nectria haematococca*, which provides resistance to an antibiotic produced by the plant host (Miao *et al.* 1991). In addition, avoidance of meiotic loss by eluding meiosis (as the PSR) or by regularization of meiotic behaviour (as appears to occur in *T. albitarse*) also leads to B chromosome maintenance, although only the latter mechanism is able to integrate B chromosomes as members of the A genome.

We thank M. J. P. Araújo for his assistance in the collection of specimens, L. Beukeboom and T. Sharbel for critical reading of the manuscript and useful comments and S. T. P. Amarantes (Zoology Museum of the Universidade de São Paulo) for his valuable information on the taxonomy of *T. albitarse*. This study was supported in part by grants from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Coordenação de Aperfeiçormento de Pessoal de nível Superior and Conselho Nacional de Pesquisa (S.M.S.R.A and S.G.P.) and Ministério de Ciencia y Tecnología (BOS2000-1521) and Junta de Andalucía (CVI-165) (F.P. and J.P.M.C.).

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