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RESEARCH ARTICLE

B chromosome ancestry revealed by histone genes in the migratory locust

María Teruel · Josefa Cabrero · Francisco Perfectti · Juan Pedro M. Camacho

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Abstract In addition to the standard set of chromosomes (A), about 15% of eukaryote genomes carry B chromosomes. In most cases, B chromosomes behave as genomic parasites being detrimental for the individuals carrying them and prospering in natural populations because of transmission advantages (drive). B chromosomes are mostly made up of repetitive DNA sequences, especially ribosomal DNA (rDNA), satellite DNA and mobile elements. In only two cases have B chromosomes been shown to carry protein-coding genes. Although some B chromosomes seem to have derived from interspecific hybridisation, the most likely source of B chromosomes is the host genome itself, but the specific A chromosome being the B ancestor has not been identified in any B-containing species. Here, we provide strong evidence for B chromosome ancestry in the migratory locust, based on the location of genes for the H3 and H4 histones in the B chromosome and a single A chromosome pair (i.e. the eighth in order of decreasing size). The high DNA sequence similarity of A and B chromosome H3–H4 genes supports B-origin from chromosome 8. The higher variation shown by B sequences, compared to A sequences, suggests that B chromosome sequences are most likely inactive and thus less subjected to purifying selection. Estimates of time of divergence for histone genes from A and B chromosomes suggest that B chromosomes are quite old (>750,000 years), showing the B-chromosome ability to persist in natural populations for long periods of time.

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M. Teruel · J. Cabrero · F. Perfectti · J. P. M. Camacho (✉)
Departamento de Genética, Universidad de Granada,
18071 Granada, Spain
e-mail: jpmcamac@ugr.es

Introduction

B chromosomes may arise intraspecifically from their host A chromosomes, or else interspecifically through hybridisation (Camacho et al. 2000; Jones and Houben 2003; Camacho 2005), but their detailed ancestry is very difficult to uncover due to the high mutability of these dispensable elements (López-León et al. 1993; Bakkali and Camacho 2004). In human beings, the presence of supernumerary marker chromosomes is frequently reported, which, in most cases, have been shown to derive from one of the human A chromosomes (Fuster et al. 2004; Liehr et al. 2008). These extra chromosomes are young and their precise origin can be traced back with the powerful tools that molecular cytogenetics provides in our species. In other organisms, however, extra chromosomes becoming B chromosomes usually undergo major transformations (e.g. amplification of repetitive DNA sequences, chromosome rearrangements, etc.), yielding Bs that can actually be a puzzle of DNA sequences coming from several A chromosomes whose precise origin is difficult to uncover (Jamilena et al. 1995; Dhar et al. 2002; Jones and Houben 2003).

Molecular studies in the last two decades have shown that B chromosomes in both plants and animals mostly contain repetitive DNAs, such as ribosomal DNA (rDNA), centromeric DNA, telomeric DNA and mobile elements (for review, see Camacho 2005). In only two cases, i.e. the fungus *Nectria haematococca* (Miao et al. 1991) and

several Canidae species (Graphodatsky et al. 2005; Yudkin et al. 2007), protein-coding genes have been found in B chromosomes.

The migratory locust (*Locusta migratoria*) harbours a very widespread B chromosome system being present in natural populations from all its geographical range: Japan (Itoh 1934; Nur 1969; Kayano 1971), China (Hsiang 1958), Mali (Dearn 1974), Australia (King and John 1980) and Spain (Cabrero et al. 1984). These B chromosomes are mitotically unstable due to non-disjunction during embryonary mitoses, which constitutes a premeiotic drive mechanism in males of this species (Nur 1969; Cabrero et al. 1984; Viseras et al. 1990; Pardo et al. 1994, 1995). In addition, these B chromosomes show drive through females due to preferential migration of B chromosomes to the oocyte (Pardo et al. 1994).

Cytological analysis showed that the proximal half of this B chromosome is euchromatic and the distal half is heterochromatic (Cabrero et al. 1984; Santos 1980). Fluorochrome banding showed that the distal heterochromatin of the B is $DAPI^+$, and thus contains A+T-rich DNA, whereas a small proximal region is $CMA3^+$, thus containing G+C-rich DNA (Camacho et al. 1991). We have recently performed the microdissection and chromosome painting of X and B chromosomes in this species, which has suggested the intraspecific origin of B chromosomes (Teruel et al. 2009). In order to increase the knowledge of molecular content and origin of B chromosomes, we present here the chromosome mapping of several repetitive DNA sequences (i.e. 45S and 5S rDNA, telomeric DNA, and H3–H4 histone genes) in A and B chromosomes, as well as the PCR amplification of these DNA sequences on 0B genomic DNA and B-DNA obtained by B chromosome microdissection. Only the H3–H4 histone genes showed to be present in the B chromosome. We cloned and sequenced part of these genes from microdissected B chromosome DNA and 0B genomic DNA and compared DNA sequence from A and B chromosomes.

Materials and methods

Five males and four females of *L. migratoria* were collected at Las Gabias (Granada, Spain) in October 2006, with which a laboratory culture was started. To obtain embryos for chromosome analysis, egg-pods were incubated at 28°C for 6 days, and the embryos were dissected out of the eggs and fixed in 3:1 ethanol–acetic acid after colchicine and hypotonic treatments. Details on procedures for embryo fixation and how cytological preparations were performed are given in Camacho et al. (1991).

We extracted genomic DNA from *L. migratoria* 0B males (gDNA) and obtained B-chromosome DNA by microdissection (μB-DNA; see Teruel et al. 2009). We then tried to amplify, by PCR, several repetitive DNAs (45S and 5S rDNA, and H3 and H4 histone genes) in both gDNA and μB-DNA, in order to test for their presence in the B chromosome.

The 45S rDNA was amplified using the 18S and ITS4 universal primers (White et al. 1990), which are anchored in the 18S and 28S genes, respectively, and amplify a DNA fragment including ITS1, 5.8S rDNA and ITS2, and the 18S and 28S flanking regions. PCR was performed in 1× PCR buffer (MBL), 2 mM Cl_2Mg, 200 μM dNTPs, 10 μM of every primer, 1 U of Taq DNA polymerase (MBL) and 100 ng of genomic DNA. PCR conditions included an initial denaturation at 95°C for 5 min and then 30 cycles at 94°C (30 s), 55°C (30 s) and 72°C (30 s) plus a final extension at 72°C for 7 min.

The 5S rDNA was amplified with the 5S-nRNA.F-1 (5′AAC GAC CAT ACC ACG CTG AA-3′) and 5S-nRNA.R-1 (5′-AAG CGG TCC CCC ATC TAA GT-3′) primers, designed from the 5S rRNA sequence in *Drosophila melanogaster* (see Cabrero et al. 2003). PCR was performed as described above, excepting 52°C hybridisation temperature.

The H3 histone genes were amplified with the primers H3aF, 5′-ATG GCT CGT ACC AAG CAG ACV GC, and H3aR, 5′-ATA TCC TTR GGC ATR ATR GTG AC (Colgan et al. 1998). PCR was performed as described above, but a final concentration of 20 μM for every primer was used. PCR conditions were 5 min DNA denaturation at 95°C, 30 cycles at 94°C (15 s), 48°C (15 s) and 72°C (15 s), and a final extension at 72°C for 7 min. H4 histone genes were amplified with universal primers (H4F2s, 5′-TSC GIG AYA ACA TYC AGG GIA TCA C and H4F2er, 5′-CKY TTI AGI GCR TAI ACC ACR TCC AT), which yielded PCR products of 211 bp (see Pineau et al. 2005). PCR was performed as described above for the 45S rDNA, in the following conditions: initial denaturation at 95°C for 5 min and then 30 cycles at 94°C (20 s), 58°C (20 s) and 72°C (30 s) plus a final extension at 72°C for 7 min.

All PCR products were visualised in a 1.5% agarose gel after adding 1:1,000 SYBR safe®.

Only the H3 and H4 genes did amplify from the μB-DNA, so that we sequenced the PCR products and confirmed that they were histone genes in a Blast search. We then cloned the PCR-obtained bands for these genes, from both gDNA and μB-DNA, by linking them to a TOPO TA cloning vector, and cloned in One Shot® TOP10 Competent Cells. A number of clones (32 for H3 and 28 for H4) were chosen for DNA sequencing. We then isolated the plasmid DNA with the Perfectprep® Plasmad Mini kit (Eppendorff) and sequenced it in both directions in either the Service of Genetic Analysis of the Department of Genetics (University of Granada) or Macrogen Inc.

(Korea). All sequenced DNAs were identified as H3 or H4 histone genes by Blast search.

We edited DNA sequences with Bioedit v.7.9.0 (Hall 1999) and made sequence alignments with ClustalW (Thompson et al. 1994) implemented in Bioedit. For DNA sequence analyses, we discarded the primer regions at both ends. DNA diversity analyses were performed with the DnaSP software (Librado and Rozas 2009). Evolutionary divergence analyses and selection tests for H3 and H4 sequences were performed with the MEGA software (Tamura et al. 2007) using the Nei–Gojobori method (Nei and Gojobori 1986). Statistical comparisons between DNA sequence parameters were performed by the Student *t* test.

The DNA sequence data from this study have been submitted to GenBank under accession nos. GU111931–GU111938 (H3 from *L. migratoria* 0B genomic DNA), GU111939–GU111948 (H3 from *L. migratoria* B chromosome DNA), GU111949 (H3 from *Eyprepocnemis plorans* 0B genomic DNA), GU111950 (H4 from *L. migratoria* 0B genomic DNA), GU111951–GU111956 (H4 from *L. migratoria* B chromosome DNA), and GU111957 (H4 from *E. plorans* 0B genomic DNA).

Fluorescent in situ hybridisation (FISH) was made as described previously (Cabrero et al. 2003). We used five different DNA probes: 45S and 5S rDNA, telomeric DNA, and H3 and H4 histone genes DNA. As telomeric DNA probe, we used $(GGTTA)_7$ and $(TAACC)_7$ synthetic deoxyoligomers (Meyne et al. 1995). For the other repetitive DNAs, we generated DNA probes for FISH from the PCR products (see above) marked by nick-translation with rhodamine-11-dUTP or fluorogreen-11-dUTP. Ten or more cells were analysed for each marker in each of five embryos. Images were captured on an Olympus microscope equipped with the DP70 cooled digital camera, and plates were mounted with The Gimp freeware.

Results

FISH analysis indicated that 45S rDNA is located on chromosomes 2, 6 and 9 (see also Cabrero and Camacho 2008), but it is absent from B chromosomes (Fig. 1a). Telomeric DNA was located on both ends at each chromosome, including B chromosomes (Fig. 1b). The 5S rDNA was located in chromosomes 3 (two interstitial clusters), 9 (two interstitial clusters) and 11 (close to the centromere), but B chromosomes did neither contain this DNA (Fig. 1g,h). These results indicated that B chromosomes did not appear to have derived from chromosomes 2, 3, 6, 9 and 11.

On the other hand, H3 histone genes were interstitially located in chromosome 8 and proximally in the B chromosome (Fig. 1c,g). Double FISH with the H3 and H4 DNA probes revealed that both histone genes colocalize in chromosomes 8 and B (Fig. 1c–e). Double FISH with the 5S rDNA and H3 DNA probes showed that these markers are located on different chromosomes (Fig. 1f–h).

We then tried to amplify these three kinds of repetitive DNA (5S and 45S rDNA and partial histone H3 and H4 genes) on genomic DNA isolated from 0B individuals (0B-gDNA), and also from B chromosome DNA obtained by microdissection (Teruel et al. 2009; μB-DNA). All repetitive DNAs amplified from 0B-gDNA, but only the H3 and H4 histone genes amplified from the B-chromosome DNA. PCR products for H3 and H4 were cloned and sequenced, showing homology with GenBank H3 and H4 sequences.

The partial H3 DNA sequence analysed comprised 331 nucleotides, from codon 8 to codon 117. This DNA sequence contained 67.6% G+C, and showed higher variation for the μB-DNA since all ten clones were different in contrast with the 22 clones from 0B-gDNA which constituted only eight haplotypes (see Table 1 and Supplemental Fig. 1). Nucleotide diversity per site (π) in the ten μB-DNA sequences (0.00967±0.00222) was five times higher than that in the 22 0B-gDNA sequences (0.00192±0.00066; Student t=3.35, df=30, P=0.0011). The mean divergence between the μB-DNA and the 0B-gDNA sequences, measured as the number of substitutions per site, was actually low (0.0058±0.0012). This high similarity suggests common descent of H3 DNA sequences in the B chromosome and chromosome 8.

The analysis of the putative amino acid sequence revealed almost three times more synonymous substitutions per synonymous site (dS) in the μB-DNA (0.0097± 0.00465) than in the 0B-gDNA (0.0033±0.00181), but the difference did not reach significance (t=1.28, df=30, P= 0.105). On the other hand, the number of non-synonymous substitutions per non-synonymous site (dN) was 6.56 times higher in the μB-DNA (0.00754±0.00268) than in the 0B-gDNA (0.00115±0.00065; t=2.32, df=30, P=0.0137). Consequently, the μB-DNA showed 1.78 amino acid substitutions per sequence, a figure higher than that showed by the 0B-gDNA (0.27). One of these substitutions in a B-derived sequence (haplotype no. 8) involved position 11, where serine was substituted by phenylalanine. The former amino acid is subjected to epigenetic modification in both humans (see Uniprot database, P68431) and *Drosophila* (P02299). In addition, three out of the ten μB-DNA sequences carried a mutant stop codon, whereas this was not observed in any of the 22 0B-DNA sequences (see Supplemental Fig. 2). A comparison of the *L. migratoria* H3 haplotypes with those obtained in the grasshopper *E. plorans* (Teruel 2009), by the Nei–Gojobori neutrality test, provided significant evidence for purifying selection (P< 0.05 in all cases). When the test was performed to the *L.*

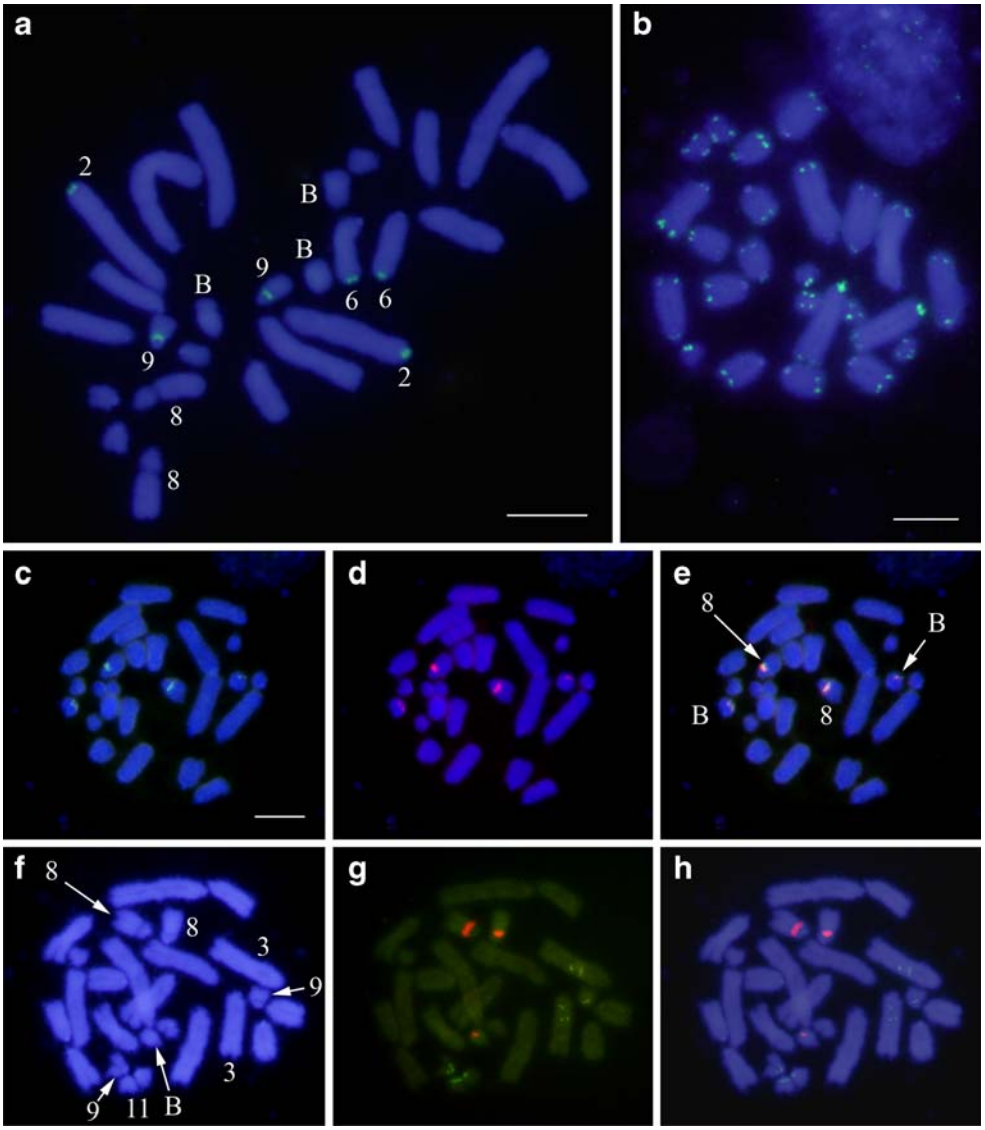

Fig. 1 Embryo cells of *L. migratoria* submitted to simple (**a**,**b**) or double (**c**–**h**) FISH for several repetitive DNA probes. **a** 45S rDNA, **b** telomeric DNA, H3 **c** +H4 **d** histone genes, **f**–**h** 5S rDNA+H3 histone gene. Note in **g** that both markers are located on different chromosomes. *Bars* represent 5 µm

migratoria haplotypes only, no departures from neutrality were observed. Finally, the d*N*/d*S* ratio was 2.23 times higher in the µB-DNA (0.78) than in the 0B-DNA (0.35). This suggests the possibility that purifying selection is relaxed for the H3 genes located in the B chromosomes, as it would expected if Bs were genetically inactive. This is also suggested by the significantly higher number of non-synonymous substitutions in the B chromosome H3 (see above).

In the case of the partial H4 DNA sequence, we analysed 58 codons (#28–#85) in 19 0B-gDNA sequences and nine µB-DNA sequences. These DNA sequences were also rich in G+C (67.8%). The genomic H4 was clearly more conserved than H3 since all 19 0B-gDNA sequences were identical, and the nine µB-DNA sequences actually constituted six haplotypes (Table 2). Nucleotide diversity per site (π) was thus zero in the 0B-gDNA and 0.00638± 0.00154 in the nine µB-DNA sequences. The mean

Table 1 Polymorphism for the H3 histone gene DNA sequences analysed from 0B genomic DNA (gDNA) and B-chromosome DNA obtained by microdissection (µB DNA)

Item	Number of sequences	Number of haplotypes	Number of sites	Number of variable sites	Number of mutations	G+C
gDNA	22	8	331	7	7	0.677
µB DNA	10	10	331	16	16	0.674
All	32	17	331	23	23	0.676

Table 2 Polymorphism for the H4 histone gene DNA sequences analysed from 0B genomic DNA (gDNA) and B-chromosome DNA obtained by microdissection (μB DNA)

Item	Number of sequences	Number of haplotypes	Number of sites	Number of variable sites	Number of mutations	G+C
gDNA	19	1	174	0	0	0.678
μB DNA	9	6	174	5	5	0.679
All	28	6	174	5	5	0.678

divergence between the μB-DNA and the 0B-gDNA sequences, measured as the number of substitutions per site, was actually low (0.00319±0.00138), which suggests common ancestry for H4 DNA sequences in the B chromosome and autosome 8 (see Supplemental Fig. 3).

There were also few differences between μB-DNA and 0B-gDNA for d*S* or d*N* (0.00232±0.00230 and 0.00353± 0.00165, respectively). Remarkably, the μB-DNA showed 0.89 amino acid substitutions per sequence, a figure quite high since all 19 DNA sequences from the 0B-gDNA were conserved, at least in the gene window analysed (see Supplemental Fig. 4). None of the observed substitutions in the B chromosome H4 involved positions being important for epigenetic modifications, as deduced from the available information in the Uniprot database (see P62805 for humans and P84040 for *Drosophila*). We found strict neutrality when *the L. migratoria* haplotypes were analysed separately (Nei–Gojobori neutrality test, $P>0.05$) but evidences of purifying selection when H4 DNA sequences from *E. plorans* were included in the analysis (Nei–Gojobori neutrality test, $P\leq0.05$). The high d*N*/d*S* ratio for the μB-DNA H4 sequences (1.72) suggests that purifying selection is also relaxed for the H4 genes located in the B chromosomes.

An estimation of the age of B chromosomes can be made following the procedure for dating gene duplications described in Li (1997) and used by Lamb et al. (2007) to estimate the age of maize B chromosomes. Following this procedure, time of divergence (T) would be the quotient between the average number of substitutions per nucleotide site between the A chromosome and B chromosome DNA sequences (K) and two times the rate of substitution for this kind of DNA sequence (r): $T=K/2r$, assuming similar substitution rate in both A and B chromosome branches. However, since both A and B branches do not appear to show similar substitution rates (Fig. 2), a more accurate estimate, especially for H4, would be to assume that all changes have taken place in the B chromosome branch only, in which case $T=K/r$. In addition, since histone genes are subjected to strong purifying selection and probably do not undergo concerted evolution (Nei and Rooney 2005) and B chromosomes in *L. migratoria* show signs of being inactive (see above), it is most appropriate to use the synonymous substitution rate of H3 and H4 for this calculation, and to assume that all the substitutions found into the B-chromosome histone genes are neutral. For this purpose, we assumed that the synonymous rate of substitution in grasshopper histone H3 and H4 genes is roughly similar to those previously shown for these genes in mammals (4.52×10^{-9} for H3 and 3.94×10^{-9} for H4, per nucleotide site and per year; see Li 1997). The observed substitutions per site mean (K) between A and B sequences was equal to 0.00490 for H3 and 0.00320 for H4. This provided estimates for time of divergence between A and B chromosome DNA sequences of 1.084 Myr for H3 and 0.811 Myr for H4.

Discussion

One of the most difficult challenges of B chromosome research is to ascertain which specific A chromosome was the ancestor of a B chromosome. Only a few researches have got close to a solution. For instance, DNA sequence comparisons in the New Zealand frog *Leiopelma hochstetteri* revealed that the B chromosome appears to be derived from the W sex chromosome (Sharbel et al. 1998). In *Nasonia* wasps, a neo-B chromosome emerged in experimental interspecific crosses, and the presence of a colour-marker gene indicated that it presumably derived from chromosome 4 (Perfectti and Werren 2001). In the plant *Plantago lagopus*, the origin of a heterochromatic neo-B chromosome from chromosome 2 was witnessed in an experimental cross, and this implied aneuploidy, the formation of a ring chromosome, chromosome fragmentation, massive amplification of 5S rDNA and centromere misdivision with chromatid nondisjuntion (Dhar et al. 2002). In maize, most DNA sequences found in B chromosomes are also present in A chromosomes, including many centromeric DNA sequences, which supports the hypothesis that Bs may be a degenerated form of an A chromosome (Lamb et al. 2005). One of these sequences (*Cent4*) seems to be shared only by chromosome 4 and the B, suggesting an evolutionary relationship of both chromosomes (Page et al. 2001). Jones and Houben (2003) have suggested an interesting pathway for B chromosome origin for micro-B chromosomes in the plant *Brachychome dichromosomatica*, specifically that they are a conglomerate

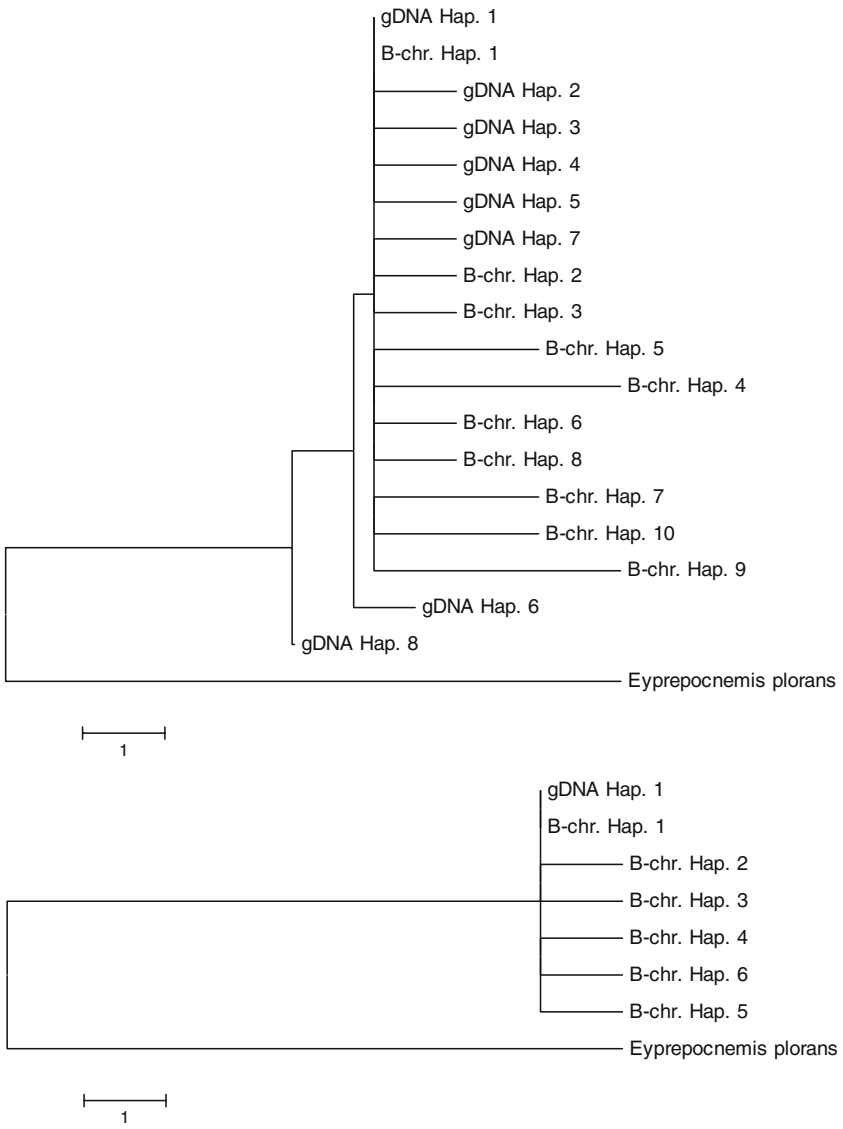

Fig. 2 Relationship among 0B genomic (gDNA) and B-chromosome (B-chr.) derived H3 (*upper tree*) and H4 (*lower tree*) DNA sequences, inferred by the neighbour-joining method (Saitou and Nei 1987). The trees are drawn to scale, with branch lengths expressed in number of substitutions

of repetitive sequences, released from different A chromosomes, that were stabilised by the addition of telomeric repeats and other sequences.

The fact that most DNA sequences hitherto isolated from B chromosomes are non-coding repetitive DNAs, excepting rDNAs and mobile elements, usually located on multiple A chromosomes, makes it difficult to ascertain a precise B chromosome ancestry in most cases. In *L. migratoria*, however, the presence of H3–H4 DNA clusters restricted to the B chromosome and the eighth autosome in size provides strong support to B chromosome ancestry from this autosome. This is actually the first demonstration for the presence of the histone multigene family in a B chromosome. By constituting a clustered gene family, histone genes could be an interesting marker for investigating B chromosome origin in other B chromosome systems.

In sharp contrast with the high variation among grasshopper species shown by 45S rDNA (Cabrero and Camacho 2008), H3 and H4 histone genes show an extreme conservatism in chromosome location since they are located in the eighth-sized autosome in all 11 grasshopper species, including *L. migratoria*, belonging to the subfamily Oedipodinae, analysed by Cabrero et al. (2009). Fossil record shows that the Oedipodinae subfamily is at least 15 million years old (see Stidham and Stidham 2000). In fact, the age of the Oedipodinae subfamily has been estimated to be about 120 million years by mtDNA molecular phylogenetic analysis (Chapco et al. 2001) and also by the association between geographical distribution of Acrididae subfamilies and the age of particular tectonic events (Jago 1983). The absence of differences in chromosome location for H3–H4 histone genes among oedipodine species indicates an extremely high conservatism of this trait in this old grasshopper subfamily, and provides strong support to B chromosome ancestry from chromosome 8, since the most simple explanation for the presence of a cluster of

histone genes in the B chromosome is that the B derived from chromosome 8, i.e. the only A chromosome carrying a cluster of histone genes. Alternatively, we cannot rule out the possibility that the histone gene cluster in the B might have derived from an orphon-like gene(s) in any other A chromosome and subsequent amplification. Childs et al. (1981) reported the existence of orphons (solitary genetic elements derived from tandem multigene families) for histone genes in the sea urchin (*Lytechinus pictus*) and the fruit fly (*D. melanogaster*). The possibility thus exists that the B chromosome in *L. migratoria* would have derived from an A chromosome other than 8 but carrying some orphon-like copies (below the FISH sensitivity limit), but this hypothesis is less parsimonious than the origin from chromosome 8 since it needs interchromosomal histone gene movement and further amplification of the histone genes in the B chromosome to surpass the FISH sensitivity limit, two facts that do not seem to have occurred in most grasshopper species (Cabrero et al. 2009).

Some of the H3 and H4 DNA sequences isolated from *L. migratoria* B chromosomes showed the same putative amino acid sequence as those obtained from 0B genomic DNA, thus suggesting that they are potentially active. In addition, some B chromosome DNA sequences carried a stop codon or non-synonymous substitutions leading to amino acid substitutions, suggesting that they were defective. It will be interesting to investigate whether these genes in the B are active, thus contributing a gene function being helpful for the host genome. The expression of these B-chromosome genes would be considered as a mutualistic phenomenon and would have important consequences on the evolutionary dynamics of a parasitic B chromosome showing drive through both males and females (see Pardo et al. 1994).

Our estimate of divergence time based on H3 and H4 sequences suggests that B chromosomes in *L. migratoria* are quite old (likely more than 750,000 years old), which is not unexpected given the worldwide distribution of B chromosomes in this species (see "Introduction" section). In a plague locust, with high capability to flight, a long time of existence assures the possibility for a B chromosome to reach every corner in the species' geographical range. Such a long existence can be granted by the highly parasitic nature of these B chromosomes, which show drive in both sexes, i.e. meiotic drive in females and premeiotic drive in males (see Pardo et al. 1994). Although female drive might not be present in all populations analysed (see, for instance, Cabrero et al. 1984) thus showing signs of possible B chromosome neutralisation (see Camacho et al. 1997), male drive has been shown in populations as distant as Japan (Nur 1969; Kayano 1971) and Spain (Cabrero et al. 1984; Viseras et al. 1990). With a drive mechanism present in such a widespread distribution area, the invasive ability of these B chromosomes is warranted, explaining their long existence and worldwide distribution.

The age of B chromosomes has previously been estimated in only two cases, i.e. the grasshopper *Myrmeleotettix maculatus* and maize. In *M. maculatus*, the absence of B chromosomes in populations from continental Europe and their presence in the British Islands suggest that this B chromosome system arose in Britain after the separation from mainland Europe about 8,000–10,000 years ago (Hewitt and Ruscoe 1971). In the case of maize, Lamb et al. (2007) have estimated a minimum age of 2 million years for the B chromosome, based on the LTR divergence of retroelements interrupting the B-specific sequences. As these authors pointed out, this long time indicates that "B chromosomes can persist in populations through a time-scale on the same order of magnitude as speciation". In fact, in both Acridid grasshoppers and rats, closely related species belonging to a same genus share B chromosomes showing highly similar characteristics and thus suggesting common descent (see Camacho 2005). Maize B chromosomes show several drive mechanisms and scarce harmful effects on carrier plants (see Puertas 2002). Likewise, *L. migratoria* Bs show drive in both sexes (Pardo et al. 1994) and are scarcely harmful for carriers (Castro et al. 1998). It thus seems that the combination of prudence in effects and aggressive behaviour in transmission does provide long life for B chromosomes.

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