Cytogenetic and Genome Research

Possible Introgression of B Chromosomes between Bee Species (Genus *Partamona*)

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Abstract

The origin of supernumerary (B) chromosomes is still a debated topic, with intra- and interspecific origins being the most plausible options. In the bee *Partamona helleri*, a sequence-characterized amplified region (SCAR) marker being specific to B chromosomes suggested the possibility of interspecific origin. Here, we search for this marker in 3 close relative species and perform DNA sequence comparison between species. The SCAR sequence does not show homology with other sequences in the databases, but does contain an open reading frame with sequence homology with a reverse transcriptase. Dot-blot hybridization using the SCAR marker as a probe confirmed that it is present in B-carrying, but not B-lacking larvae of *P. helleri*, and indicated its presence in adult individuals of *P. cupira* and *P. criptica*. Additionally, PCR amplification of the SCAR marker was successful on genomic DNA obtained from *P. helleri* and *P. rustica* larvae carrying B chromosomes, and on genomic DNA obtained from adult individuals of *P. cupira*, *P. criptica* and *P. rustica*. Finally, a comparison of the DNA sequence of the SCAR markers amplified from these 4 species showed very few nucleotide differences between the species. The complete association between B chromosome and SCAR presence and the scarce divergence observed for this DNA sequence between the 4 species analyzed suggest the possibility that this B chromosome has recently been transferred between species through several episodes of interspecific hybridization.

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B chromosomes are additional dispensable chromosomes that likely originated from the standard (A) chromosomes but show non-Mendelian transmission modes and follow their own evolutionary pathway [Camacho et al., 2000; Camacho, 2005]. Since being discovered by Wilson [1907], B chromosomes have remained mysterious elements with no known origin or role. However, the introduction of molecular genetic and cytogenetic techniques in the last 2 decades has provided much insight

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regarding several aspects of B chromosome biology [Camacho et al., 2000; Camacho, 2004, 2005; Masonbrink et al., 2012].

Based on molecular studies, 2 hypotheses have been proposed to explain the origin of B chromosomes, both suggesting their origin from A chromosomes but differing in whether the A chromosomes belong to the same species harboring the B chromosomes (the intraspecific hypothesis) or to a different species (the interspecific hypothesis). The intraspecific origin is suggested by the absence of B-specific DNA sequences, but there are also B chromosomes that have arisen intraspecifically (e.g. those in rye) containing specific satellite DNA repeats formed de novo from A chromosome DNA sequences [Langdon et al., 2000]. Other examples of intraspecifically derived Bs have been reported in the animals *Chironomus plumosus*, *Drosophila subsilvestris*, *Petauroides volans*, *Reithrodontomys megalotis*, *Eyprepocnemis plorans*, several *Glossina* species, and *Leiopelma hochstetteri* and in the plants *Crepis capillaris* and *Brachychome dichromosomatica* [for a review, see Camacho, 2005]. Recently, next-generation sequencing has revealed the intraspecific origin of B chromosomes in rye [Martis et al., 2012].

The interspecific origin of B chromosomes is suggested by the presence of DNA sequences that are absent in the A chromosomes of the same species but are present in the A chromosomes of a closely related species. Some examples have been described in plants of the genus *Coix* [Sapre and Desphande, 1987], the gynogenetic fish *Poecilia formosa* [Schartl et al., 1995] and the wasp *Nasonia vitripennis* [McAllister and Werren, 1997; Perfectti and Werren, 2001].

The bee *Partamona helleri* (Hymenoptera, Apidae) is abundant in southeast Brazil, from the north of Santa Catarina state to the south of Bahia state [Pedro and Camargo, 2003]. This haplodiploid species has 34 chromosomes in females and 17 in males. In addition, individuals with up to 4 B chromosomes have been found [Costa et al., 1992; Brito et al., 1997; Tosta et al., 2004]. Previous studies have shown that B chromosomes are widespread in Brazilian populations of *P. helleri* and that they are mostly heterochromatic and contain AT-rich regions [Brito et al., 1997, 2005; Brito-Ribon et al., 1999]. Tosta et al. [2004] isolated, cloned and partially sequenced a random amplified polymorphism DNA (RAPD) marker associated with B chromosome presence. From this RAPD marker, Tosta et al. [2007] developed a sequence-characterized amplified region (SCAR) marker that showed complete association with B chromosome presence in *P. helleri*, as demonstrated by simultaneous molecular and cytogenet-

ical analyses. This marker is useful to ascertain the presence of the B chromosome in adult individuals because cytogenetic techniques for visualizing chromosomes in adult bees are not available.

Recently, Martins et al. [2013] performed chromosome painting with B-specific DNA probes obtained through microdissection of the *P. helleri* B chromosome and failed to demonstrate hybridization of the B probe on the A chromosomes of this species, suggesting the possible interspecific origin of this B chromosome. In addition, Marthe et al. [2010] showed that the *P. helleri* SCAR marker is present in B-carrying larvae but absent in B-lacking ones of *P. cupira*.

Here, we analyze the presence of the SCAR marker in 4 *Partamona* species and compare their DNA sequences. Our results indicated that the SCAR marker is present in *P. helleri* and *P. rustica* larvae carrying B chromosomes but absent in larvae lacking B chromosomes. In addition, we show that the SCAR marker is also present in some adult individuals of *P. cupira* and *P. criptica*. We finally performed a comparative analysis of the SCAR DNA sequence which showed a remarkably low number of nucleotide differences between the 4 species.

Materials and Methods

Materials and Cytological Analysis

Four species of the bee genus *Partamona* were analyzed for the present research. Fifty larvae from 5 nests of *P. helleri* were obtained from cultures maintained in the Universidade Federal de Viçosa from samples originally collected at Pedra do Anta and São Miguel do Anta [Viçosa, Minas Gerais (MG), Brazil]. In addition, we collected 3 adult specimens of *P. cupira* at Guimarânia (MG, Brazil), 3 adults of *P. criptica* at Domingos Martinho (Espírito Santo, Brazil), 3 adults of *P. rustica* at Januária (MG, Brazil), and 60 larvae of *P. rustica* from 6 nests at Lontra (MG, Brazil).

Chromosome preparations were made according to Imai et al. [1988] from cerebral ganglia cells of postdefecating larvae. To score the number of B chromosomes in each larva, 10 mitotic metaphase cells were analyzed under a BX-60 Olympus microscope.

Fluorescent in situ Hybridization

We performed FISH according to Viégas-Péquinot [1992] with some modifications, and, in the case of the SCAR marker, we also tested the protocol reported in Cabrero et al. [2003a, b]. Two DNA probes were labeled, one using 1 μg of the cloned RAPD-DNA (clone P1) [Tosta et al., 2004] containing the pGEM-T easy recombinant plasmid and another using 1 μg of the *D. melanogaster* pdm 238 rDNA (18S, 5.8S and 28S plus intergenic sequences) [Roiha et al., 1981] inserted into the pBR 32 plasmid. The labeling reaction was performed with the BIONICK-GIBCO Nick-Translation kit. Each chromosome preparation previously selected for FISH was treated with 150 μl of RNAse (100 μg/μl), covered with a coverslip and incubated in a humid chamber at 37°C for 1 h.

The preparations were rinsed 3× at room temperature in 2× SSC for 3 min and then dehydrated in 50 and 75%, and absolute ethanol (3 min each) at room temperature. Once dried, the preparations were submitted to 70% formamide for 2 min at 70°C for DNA denaturation. The probe was added to the hybridization mixture, denatured at 100°C for 10 min, placed on ice, and centrifuged for 30 s at 12,000 rpm. Simultaneously, the chromosome preparations were rinsed 3× in cold 2× SSC (2 min each) and then dehydrated in an ethanol series (50 and 75%, and absolute, 2 min each). Once the preparations were dried, 10 μl of the probe was added, and the samples were covered with a 24 × 24 plastic coverslip and incubated overnight at 37°C. After 1.5 days, the preparations were immersed twice in 50% formamide at 37°C (2 min each).

For probe detection, the preparations were immersed twice in PBT at room temperature (5 min each). Once dry, 100 μl of anti-biotin antibody, composed of 0.6 μl of streptavidin-Alexa Fluor® 488 conjugate (Life Technologies) and 99.4 μl of PBT, was added to each preparation, and the preparations were covered with a 24 × 32 glass coverslip. The preparations were incubated in a humidified Petri dish with PBT at 37°C for 45 min and then rinsed twice in PBT at room temperature (5 min each). A 1% anti-IgG solution in PBT (1 μl of anti-IgG: 99 μl of PBT) was centrifuged for 5 min at 4°C and 12,000 rpm. After adding 100 μl of anti-IgG solution, the preparations were covered with a 24 × 32 glass coverslip and incubated in a humidified Petri dish with PBT at 37°C for 45 min in the dark. The preparations were incubated with 100 μl of propidium iodide (7 μg/ml) for 1 min and rinsed in PBS. Finally, once dry, 13 μl of Vectashield mounting medium was added to the preparations, which were then covered with a coverslip and analyzed with a fluorescence microscope.

Dot-Blot Hybridization
Genomic DNA was extracted from adults and larvae according to the method described by Waldschmidt et al. [1997]. Genomic DNA (1 μg) was denatured with 5N NaOH and 5N ammonium acetate (10 min each). For probe preparation, we amplified the SCAR marker developed by Tosta et al. [2007] from the pGEM-T easy vector containing the RAPD marker cloned by Tosta et al. [2004] after purification with miniprep (QIAprep Spin Miniprep, Qiagen). The probe was labeled with fluor-12-dUTP (Amersham Biosciences) according to manufacturer's instructions. We used nylon membranes (Stratagene) to perform the dot-blot hybridization. The staining was conducted with the CPD-Star™ detection system (Amersham Biosciences) after 20 min of exposure time at room temperature.

PCR Amplification, Cloning and Sequencing of the SCAR Marker
PCR amplification of the SCAR marker was performed as described in Tosta et al. [2007]. PCR products were visualized in agarose gels, and the SCAR fragment was extracted from the gel and eluted with the Wizard purification system kit (PROMEGA). The fragments were then ligated with the T4 ligase to the pGEM-T easy vector, which was used to transform DH 5α bacteria [Inoue et al., 1990]. The bacteria were plated on LB medium with ampicillin (100 μg/ml), 5-bromo-4-chloro-3-indol-β-D-galactoside (X gal) and isopropyl thio-β-galactoside and incubated at 37°C. Recombinant clones were extracted with the SNAP miniprep kit (Invitrogen) and sequenced in both directions by the Applied Biosystems (Hitachi) 3100 Avant Genetic Analyzer by Sanger's dideoxy-

nucleotide method [Sanger et al., 1977] using the M13F and M13R primers for the pGEM plasmid. DNA sequences were edited and aligned using Phred [Ewing et al., 1998], Consed [Gordon et al., 2001] and MEGA 4 [Tamura et al., 2007] software. The DNA sequences were deposited in GenBank with the accession numbers KC145261 *(P. helleri)*, KC145262 *(P. cupira)*, KC145263 *(P. criptica)* and KC145264 *(P. rustica)*.

Results

In *Partamona* bees, B chromosomes can be visualized cytologically in larvae, but not in adults. To evaluate the association between B chromosome presence and SCAR presence, we analyzed larvae of *P. helleri* and *P. rustica* by means of cytological and molecular methods. Both species show 2n = 34 standard chromosomes in females, and additionally, some individuals carried B chromosomes in 4 out of 5 *P. helleri* nests and 1 out of 6 *P. rustica* nests analyzed.

Physical mapping with the B-specific SCAR DNA-probe on *P. helleri* chromosomes failed to show any hybridization signals on the A or B chromosomes, even after using 2 different FISH protocols (results not shown). By contrast, the same protocols revealed the presence of a conspicuous rDNA cluster on B chromosomes and several A chromosomes (fig. 1). This indicates that the FISH protocols employed do actually work in this species and that the SCAR sequence does not show tandem repeat structure.

We analyzed the presence of the SCAR marker in 3 other *Partamona* species, by means of 2 molecular approaches: dot-blot hybridization and PCR amplification. As shown in figure 2A, the SCAR sequence dot-blot hybridized with genomic DNA obtained from 2 B-carrying larvae of *P. helleri* and with genomic DNA obtained from adult individuals of *P. cupira* and *P. criptica*. The SCAR probe, however, did not hybridize with 2 B-lacking larvae of *P. helleri*.

In agreement with these results, PCR experiments with the SCAR primers (fig. 2B) showed the amplification of the SCAR DNA in B-carrying larvae of *P. helleri*, but not in B-lacking ones. In addition, PCR experiments showed amplification of the SCAR marker in adult individuals of *P. cupira* and *P. criptica*. In a later assay, we observed that the SCAR marker also amplified by PCR in a *P. rustica* larva from a B-carrying nest (fig. 2C).

We then cloned and sequenced the SCAR fragments amplified in *P. helleri*, *P. cupira*, *P. criptica*, and *P. rustica* (fig. 3). The DNA sequence of the SCAR marker consists of 1,463 bp and shows 66% A/T, which is consistent with

Tosta et al.
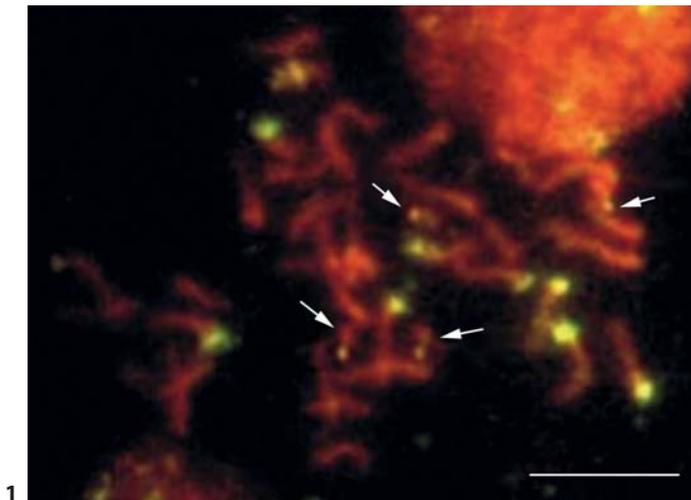
Fig. 1. FISH with the rDNA probe [Roiha, 1981] in a ganglion cell at mitotic metaphase obtained from a postdefecating larval female with 2n = 38 chromosomes. Note the presence of 4 B chromosomes (arrows) containing the rDNA in their short arms. Scale bar = 5 μm.
Fig. 2. Association of the SCAR marker with B chromosome presence in *Partamona* species. **A** Dot-blot hybridization of genomic DNA with the SCAR marker as DNA probe in *P. helleri* larvae carrying B chromosomes (a, b), *P. cupira* and *P. criptica* adults (c, d), and *P. helleri* larvae lacking B chromosomes (e, f). **B** PCR amplification of the SCAR DNA sequence in B-carrying (lane 1) and B-lacking (lane 2) larvae of *P. helleri* as well as in 3 adult individuals from *P. cupira* (lanes 3–5) and 2 adult individuals from *P. criptica* (lanes 6–7). Note the absence of the SCAR marker in an adult individual of this latter species (lane 8). **C** PCR analysis of SCAR presence in larvae of *P. rustica*. As positive controls, lanes 1 and 2 show *P. helleri* larvae from nests lacking (lane 1) and carrying (lane 2) B chromosomes. Lanes 4–8 show the absence of amplification on genomic DNA from larvae of *P. rustica* from 5 different nests (L1, L2, L4, L5 and L6) collected at Lontra (MG, Brazil), where B chromosomes were absent in the 12 larvae analyzed from each nest. Lane 9 shows SCAR DNA presence in a *P. rustica* larva from a B-carrying nest (L3) from the same population. Lane 3 is empty.
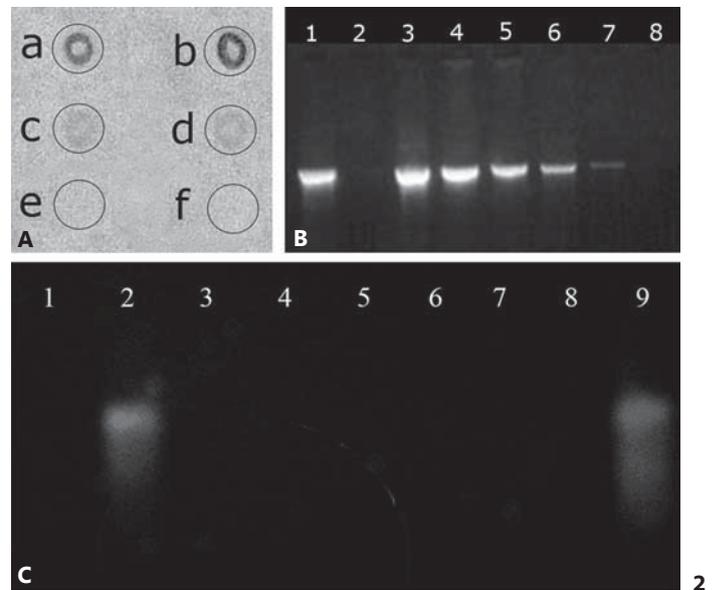
the A/T-rich nature of *P. helleri* B chromosomes reported by Brito [1998]. Megablast and discontiguous blast searches in the NCBI databank (BLASTN 2.2.12) [Altschul et al., 1997] showed no homology of the SCAR DNA sequence with any known DNA sequence. When all putative open reading frames in the *Partamona* SCAR DNA sequence were obtained and the resulting amino acid sequence was compared with the protein databases, the SCAR marker showed significant partial homology with several protein sequences, such as the predicted RNA-directed DNA polymerase from transposon BS (reverse transcriptase) in *Acyrthosiphon pisum* (a pea aphid) (XP_001945085) [score = 67.8 bits (164), expect = 2e-10, identities = 45/124 (36%), positives = 68/124 (54%), gaps = 14/124 (11%)], a predicted reverse transcriptase from a transposon X-element in the same species [score = 64.3 bits (155), expect = 3e-09, identities = 39/105 (37%), positives = 57/105 (54%), gaps = 5/105 (4%)] and an endonuclease and reverse transcriptase-like protein in *Bombyx mori* [score = 58.2 bits (139), expect = 2e-07,

identities = 40/127 (31%), positives = 67/127 (52%), gaps = 9/127 (7%)]. However, these similarities were partial and confidently ascertaining the origin and possible function of the *Partamona* SCAR sequences was difficult.

A comparison of the SCAR DNA sequences amplified from *P. helleri*, *P. cupira*, *P. criptica*, and *P. rustica* (fig. 3) indicated nearly complete identity between the 3 latter species (their sequences only differing in 4 indels of 1 nucleotide) but a difference of 5 nucleotide substitutions and 1 indel spanning a nucleotide with respect to the *P. helleri* SCAR sequence.

Discussion

To the best of our knowledge, 8 *Partamona* species had hitherto been analyzed cytologically: *P. helleri*, *P.* sp. *aff. nigrior*, *P. vicina*, *P. ayaleae*, *P. mulata*, *P. rustica*, *P.* sp. *n.* and *P. cupira* [Costa et al., 1992; Brito et al., 1997; Brito-Ribon et al., 1999; Martins et al., 2009; Marthe et al.,

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P. helleri +B   5'CCCGCTACAC ATATAATTAA AGTATAACGG TTTCTAATAG ATCTCTTTTC CCCAAAAGTA ATTATGAAGA GTAACAGAAT   80
P. cupira       5'.......... .......... C..-...... .......... .......C. A........ .......... ..........   80
P. criptica     5'.......... .......... C..-...... .......... .......C. A........ .......... ..........   80
P. rustica      5'.......... .......... C..-...... .......... .......C. A........ .......... ..........   80

P. helleri +B   TGTAGAAGAA AAAATAATTT GGAGTACTGA ATAATTTGAG CTGCCAGTGT GAAAGATAGG ATTGAATCCA ATCATATATG   160
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   160
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   160
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   160

P. helleri +B   AAATCACAGT GAAGGAGAGA GAAAAGAGAC CGATCCTATA TGATATAGAT GATATAGATG ATATAGATAA TCAAAATTAA   240
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   240
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   240
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   240

P. helleri +B   CAATTAAAAC ATTAACGAAT CGATTGAATC AGCTAGCAGA AGCTGAATAA TTAAATGAAG GGGCAAGATA CGAGCAGACA   320
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   320
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   320
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   320

P. helleri +B   GAACAACAAC GATGACTACA GTCGAGCAAG TCAAATACAG TTGAATATTT TATCTGAGTC ACTCATTGTC ATTCAATCTT   400
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   400
P. criptica     .......... .......... .......... .......... .......... -......... .......... ..........   400
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   400

P. helleri +B   CCTAACTGCT GTTTATTACC ACTCACATAT ACATTCATTA ATGTATGAAA ATTTGTAAAG ATATCCAGAC CGTCCTGGAA   480
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   480
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   480
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   480

P. helleri +B   AA-GTATGAT CATGGAAGGT ACATAAGTAC TGGCCATACC ACAAATCTGA CAAGTAGGTG CTCGTATACA TATCCGTCGG   560
P. cupira       ..-....... .......... .......... .......... .......... .......... .......... ..........   560
P. criptica     ..A....... .......... .......... .......... .......... .......... .......... ..........   560
P. rustica      ..-....... .......... .......... .......... .......... .......... .......... ..........   560

P. helleri +B   TTATGTACAT GTGTATTTAA TTTAATTTTT TTCGTTCAGA AAATATACCA ATCCTGTCAG TCAATCTTTT TAACCATTGA   640
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   640
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   640
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   640

P. helleri +B   TTATTTCTTC TCTTTTACAG CAACTCTCCT ATATTTAAAT TCAGTTAAAA CTGCGCTTAC AGATATTCTC AATGTAATAT   720
P. cupira       .......... ...G...... .......... .......... .......... .......... .......... ..........   720
P. criptica     .......... ...G...... .......... .......... .......... .......... .......... ..........   720
P. rustica      .......... ...G...... .......... .......... .......... .......... .......... ..........   720

P. helleri +B   CGGTTTATAT TGCGACCGTT AAAGACAACA AGTACCGCCT GCATAGATGG CTGTATAGGT GTTTCGAGAA GTTGGAATGT   800
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   800
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   800
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   800

P. helleri +B   CGTTGATGTA CAATATGAAG AGAAGCGGAG CAAGTATGGA TCCTTCTAGT ACCTCTGTTA TAAGGTTTCT TTATGATTGA   880
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   880
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   880
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   880

P. helleri +B   TGTGTGACTA TTTAGAATAT CTCGTGCAGA CCTAGAATTT CCTGTTTTAA CTGATGATTT TATTGATGTA TGTAGGCGGT   960
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   960
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   960
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   960

P. helleri +B   ATATTTATTT ACCGCAATTT AGTTAGAAAG ACATTGTGCC AGACGTAGGT TTTTTTTATA GGTTCAGGAA GATGAGATGT   1040
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   1040
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   1040
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   1040

P. helleri +B   GTTAGTCAGA TCTGTTATTT TTTATTACAG TGCGTTCTGT GAAACTTTAT AATTGCTGAG TGCATGAGTT TAGATCTGAA   1120
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   1120
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   1120
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   1120

P. helleri +B   TTGTGGCGAG ACTATTATTC TGGAGTTACT TAGTTGGATG AGAAAGTGAA CGTGGATGAT TTTAACTACT TTTCCTAGGA   1200
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   1200
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   1200
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   1200

P. helleri +B   TGTCTAGTAG GCTAATTGAT TTGTAATTGG AAACAGATAT TGGTATTGGA CTTATGGGTA TTCTTGGCTT TGGTATTGAA   1280
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   1280
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   1280
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   1280

P. helleri +B   CTTATTTTTG CTTCTTTCCA AGCTGCGGGA AAGTATGAAA GTTTCAGATG GGTTTTGAAT ATGTATAAAA TTTGCACAAT   1360
P. cupira       .......... .......... .......... .......... .......... .......... .......... ..........   1360
P. criptica     .......... .......... .......... .......... .......... .......... .......... ..........   1360
P. rustica      .......... .......... .......... .......... .......... .......... .......... ..........   1360

P. helleri +B   GGGTTCGGAT GGAAGCATTT TAATCTGTGG CTGTGATTTT GTCCACGTGC TTAATGAAGA GATT-CTTGA TTATGTCTCT   1440
P. cupira       .......... .......... .......... .......... .......... .......... ...T..... ..........   1440
P. criptica     .......... .......... .......... .......... .......... .......... ..-...... .-........   1440
P. rustica      .......... .......... .......... .......... .......... .......... ..-...... ..........   1440

P. helleri +B   TATCATTTAT GGAGTGCAGT TTGGGATATT TTAGGGTGTA GCGGG3'                                        1485
P. cupira       .......... .......... ....C..... .......... ....3'                                        1485
P. criptica     .......... .......... ....C..... .......... ....3'                                        1485
P. rustica      .......... .......... ....C..... .......... ....3'                                        1485
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Fig. 3. DNA sequence alignment of the SCAR marker (1,485 bp) amplified in the 4 *Partamona* species. The SCAR primers are indicated in red and blue at both ends.

Fig. 4. Geographical distribution of the 4 *Partamona* species showing the SCAR marker and constituting part of the Cupira group, according to Pedro and Camargo [2003].

2010]. B chromosomes have been found in *P. helleri* [Tosta et al., 2004, 2007; present study], *P. cupira* [Marthe et al., 2010] and *P. rustica* [present study]. In all 3 species, the SCAR sequence was only present in B-carrying nests. This association between B chromosome presence and SCAR presence was previously shown in *P. helleri* [Tosta et al., 2007] and *P. cupira* [Marthe et al., 2010], and is extended to *P. rustica* in our present work. The presence of this marker in B chromosomes from these 3 species suggests that the B chromosomes in several species of this genus might have had a common origin because either they were present in a common ancestor of these species (hypothesis 1) or the B chromosomes have been horizontally transferred between species through episodic interspecific hybridization (hypothesis 2).

Taxonomically, *P. helleri*, *P. cupira*, *P. criptica*, and *P. rustica* belong to the Cupira group within the *Partomona* genus, a group considered to be a monophyletic clade [Pedro and Camargo, 2003]. In addition, these species are sympatric (fig. 4), which provides the opportunity for intercrossing. A recent phylogenetic analysis of several *Partamona* species revealed the monophyletic origin of the species carrying B chromosomes and at least one possible hybridization event between *P. helleri* and *P. chapadicola* [Fernandes, 2012], suggesting that interspecific hybridizations may be common in this group of species. These results support the 2 hypotheses mentioned above. However, the high similarity between the SCAR DNA sequence in these 4 species would be consistent with hypothesis 1 (i.e. the B chromosomes descended from a common ancestor species) only in the case that the SCAR sequence would have strong functional constraints, which is highly unlikely for a DNA sequence located in a B chromosome, given the dispensable nature of these extra elements. By contrast, a high similarity of the B-specific SCAR sequence between species is consistent with recent introgression of B chromosomes through interspecific hybridization.

In the wasp *N. vitripennis*, the paternal sex ratio chromosome, which is the most parasitic B chromosome hitherto known, originated after hybridization with a species of the genus *Trichomalopsis*, as deduced from the DNA sequence information provided by the NATE retroelement [McAllister and Werren, 1997]. The de novo origin of B chromosomes has also been shown in experimental crosses between *N. vitripennis* and *N. giraulti* [Perfectti and Werren, 2001].

Leach et al. [2004] claimed that most B-specific DNA sequences hitherto reported are actually present in a few copies in the A chromosomes. In the fish *Alburnus alburnus*, however, it has been shown that the presence of B chromosomes composed mainly of a specific retrotransposable DNA element showing homology with the *Drosophila* Gypsy/Ty3 [Ziegler et al., 2003; Schmid et al., 2006]. This DNA sequence is very abundant in B chromosomes, but not detectable in the A chromosomes of *A. alburnus* or the B chromosomes of *Rutilus rutilus*, a close relative, suggesting that the B chromosomes in *A. alburnus* have evolved independently from the A chromosomes. Similarly, the SCAR sequence is not detectable in B-lacking individuals of *P. helleri*, *P. rustica* and *P. cupira* [Tosta et al., 2007; Marthe et al., 2010; present study]. However, if B chromosomes originated in these species through interspecific hybridization, we should not necessarily expect to find the SCAR marker in the A chromosomes of any of these species, but only in the species where the B actually arose for the first time from the host genome. In this case, the study of the SCAR presence and B chromosome presence in all *Partamona* species (and in others from related genera) would enable the identification of the species carrying the SCAR marker in the A chromosomes, thereby providing additional support for hypothesis 2. Alternatively, if the B chromosomes predated the diversification of the Cupira group of the *Partamona* species, the absence of the SCAR marker in the A chromosomes would suggest that B origin also involved interspecific hybridization in the common ancestor of these species, since the Cupira species inherited the B, but not the SCAR in the A chromosomes.

Possible Introgression of B Chromosomes

Acknowledgements

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