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ADRIANA DE SOUZA MELO

Origem e dispersão do cromossomo B de *Rhammatocerus brasiliensis* (Orthoptera - Acrididae)

Recife
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ADRIANA DE SOUZA MELO

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Tese apresentada ao Programa de Pós-Graduação em Genética da Universidade Federal de Pernambuco como parte dos requisitos exigidos para obtenção do título de Doutora em Genética.

Orientadora: Profª. Drª. Rita de Cássia de Moura

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RESUMO

O gafanhoto *Rhammatocerus brasiliensis* possui ampla dispersão no nordeste brasileiro, em especial em Pernambuco. Este estudo investigou a origem do cromossomo B de *R. brasiliensis* através do mapeamento de DNA satélite, dispersão desse cromossomo e da H3 em populações do Nordeste brasileiro. Os cariótipos de 1274 indivíduos foram analisados, em 91 destes foram mapeados DNAs satélite (DNAsat), histona H3 e o elemento *Rbra_Gypsy1*. Dezenove das 21 populações apresentaram B, com frequência de 0 a 18,8%, média de 8,5%. A heterocromatina constitutiva (HC) é pericentromérica em todos os cromossomos, incluindo o B. Em três populações foram observados um cromossomo B variante, o qual possui um bloco terminal adicional de HC. A análise molecular populacional em 200 indivíduos de 10 populações indicou fluxo gênico entre as populações, favorecendo a dispersão do B. DNAsat restrito ao par S11 e ao B foi identificado, indicando a origem autossômica intraespecífica desse cromossomo. A histona H3 revelou dois padrões de localização, conservado e disperso, que variou de moderado a amplo, sendo possível identificar o par portador do sítio ancestral da histona no bivalente M7. O mapeamento do *Gypsy* foi similar ao da H3 quando disperso. O *Rbra_Gypsy1* mostrou domínios conservados indicando ser autônomo, o que pode promover sua ação no genoma da espécie hospedeira e promover recombinação ectópica, levando assim a ampla dispersão das sequências de H3, incluindo o B. Este elemento ainda pode estar exercendo alguma função estrutural nos centrômeros da espécie. O presente estudo contribuiu para o entendimento da origem, composição e dispersão do cromossomo B da espécie *R. brasiliensis.*

Palavras-chave: Gafanhoto. Cromossomo supernumerário. Famílias multigênicas. Fluxo gênico.

ABSTRACT

The grasshopper *Rhammatocerus brasiliensis* has wide dispersion of the B chromosome and H3 histone sequences, in Pernambuco. This study investigated the origin of B in *R. brasiliensis*, and dispersion of the same and H3 in populations of Brazilian Northeast. The karyotypes of 1274 individuals were analyzed, in 91 of them satellite DNAs (satDNAs), H3 histone and the *Rbra_Gypsy1* element were mapped. Nineteen of the 21 presented B, the frequency varied from 0% to 18.8%, with a mean of 8.5%. Constitutive Heterochromatin is located in pericentromeric region on all chromosomes, including B. The variant B was observed in three populations and has an additional block terminal. A population molecular analysis of 200 individuals from 10 populations indicated the gene flow between the populations (FST 0.15 and GST 0.17), favoring a dispersion of Bs. The satDNA restricted to the S11 and B pair was found, indicating a source intraspecific form of this chromosome. H3 histone revealed two patterns of localization, conserved and dispersed, ranging from moderate to broad. It was possible to identify the of ancestral site the H3 histone. Gypsy mapping was similar to that of H3 when dispersed. *Rbra_Gypsy1* showed conserved domains indicating autonomy, which may promote its action in the genome of the host species and promote ectopic recombination, thus leading to wide dispersion of H3 sequences. This element may still be exerting some structural function in the centromeres of the species. The present data contributed to understanding of the origin, composition and dispersion of the B chromosome of the *R. brasiliensis* species.

Key words: Grasshopper. Supernumerary chromosome. Multigene families. Gene flow.

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LISTA DE ABREVIATURAS E SIGLAS

Item	Definição
ºC	Graus Celcius
2n	Número diploide
B	Cromossomo B
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
dATP	Desoxiadenosina trifosfato
DNA	Ácido desoxirribonucleico
DNAsat	DNA satélite
DNAsn U2	DNA pequeno nuclear U2
DNA C_0t-1	DNA enriquecido com sequências altamente e moderadamente repetitivas
dUTP	Desoxiuracila-trifosfato
FACEPE	Fundação de Amparo à Ciência e Tecnologia de Pernambuco
FAPESP	Fundação de Amparo à Ciência do Estado de São Paulo
G	Par cromossômico grande
H3	Histona H3
HC	Heterocromatina constitutiva
ISSR	Regiões entre sequências de repetições simples
M	Par cromossômico médio
MgCl2	Cloreto de magnésio
NCBI	*National Center for Biotechnology Information*
NGS	Sequenciamento de Nova Geração
P	Par cromossômico pequeno
PCR	Reação em Cadeia da Polimerase
rDNA	DNA ribossomal
RNA	Ácido ribonucleico
rRNA	RNA ribossomal
SOAP	Short Oligonucleotide Analysis Package
TEs	Elementos de transposição
X	Cromossomo X

SUMÁRIO

1 INTRODUÇÃO

Os cromossomos B são elementos adicionais dispensáveis do cariótipo e constituem um dos polimorfismos mais difundidos entre os eucariontes. Sua ocorrência é praticamente universal e foi observado em cerca de 15% das espécies de eucariontes analisadas. No reino animal, 76% das espécies com ocorrência de polimorfismo para cromossomos B são insetos, sendo considerado o polimorfismo mais comun na ordem Orthoptera, com aproximadamente 191 espécies portadoras de cromossomos B, constituindo 11,9% das espécies estudadas.

Estudos sobre cromossomos B classicamente abordam a ocorrência, frequência, número e localização de marcadores cromossômicos obtidos através de bandeamentos. Trabalhos recentes realizados em gafanhotos e outros organismos, têm mapeado sequências repetitivas como DNA ribossomal, DNA satélite, histona H3, DNAsn U2 e elementos transponíveis. Adicionalmente, também foram realizadas análises moleculares (análises populacionais e de composição molecular) visando revelar a origem, comportamento, composição, função das sequências e aspectos referentes a evolução dessas sequências.

O polimorfismo quanto à presença de um ou dois cromossomos B foi detectado no gafanhoto *Rhammatocerus brasiliensis* em indivíduos de várias populações de Pernambuco e Bahia. Cromossomos B da espécie também foram analisados quanto a localização e composição de heterocromatina constitutiva (HC), observada na região pericentromérica e apresentou riqueza para os pares de bases GC.

Além disso, sequências de DNAr 18S, DNAr 5S e da histona H3 foram mapeadas no cariótipo de espécimes de três populações de Pernambuco. As sequências de H3 estão dispersas em todos os cromossomos, exceto no par S11, e no B. Essa sequência encontra-se colocalizada a sequência de DNAr 5S.

Considerando a ampla dispersão do cromossomo B em *R. brasiliensis* em Pernambuco e das sequências repetitivas de histona H3 no genoma dessa espécie, propusemos: I - investigar a ocorrência do cromossomo B em populações de outros estados do Nordeste brasileiro e se há fluxo gênico entre elas; II - verificar se a origem do cromossomo B é inter ou intraespecífica; III - verificar se as sequências de histona H3 encontram-se dispersas em todas as populações analisadas e se elementos de transposição estão relacionados ao processo de dispersão.

1.1 OBJETIVOS

1.1.1 Objetivo geral

Investigar a ocorrência e composição de cromossomo B de *Rhammatocerus brasiliensis* (Orthoptera – Acrididae) e os mecanismos envolvidos na origem e dispersão destes cromossomos em populações do Nordeste do Brasil.

1.1.2 Objetivos específicos

1. Analisar a ocorrência e frequência do cromossomo B em diferentes populações de *R. brasiliensis* do Nordeste brasileiro, e verificar se há conectividade entre as populações e se a mesma estaria contribuindo para a dispersão do B.
2. Investigar a origem do cromossomo B de *R. brasiliensis* através do mapeamento de sequências repetitivas, visando determinar se ela foi inter ou intraespecífica.
3. Mapear e verificar se a dispersão de sítios de Histona H3 em *R. brasiliensis* ocorre em outras populações do Nordeste brasileiro, e sua possível relação com elementos de transposição da família *Gypsy*.

2 REVISÃO DA LITERATURA

2.1 CONSIDERAÇÕES SOBRE A TAXONOMIA E DISTRIBUIÇÃO DO GÊNERO *RHAMMATOCERUS* (ACRIDIDAE)

O gênero *Rhammatocerus* possui 18 espécies descritas (*R. alticola*, *R. brunneri*, *R. conspersus*, *R. cyanipes*, *R. gregarius*, *R. palustris*, *R. peragrans*, *R. pictus*, *R. pratensis*, *R. brasiliensis*, *R. salinus*, *R. schistocercoides*, *R. smithi*, *R. suffusus*, *R. varipus*, *R. viatorius viatorius*, *R. viatorius cyanomerus*, *R. viatorius excelsus*) com distribuição na região neotropical (ASSIS-PUJOL, 1998). Entre os gafanhotos, o gênero *Rhammatocerus* é um dos mais comuns em algumas regiões da América Tropical e Subtropical (CARBONELL, 1988) e possuem grande importância econômica por serem apontados como devastadores ocasionais (ASSIS-PUJOL, 1998; SILVA *et al.,* 2006).

Entre as espécies do gênero *Rhammatocerus*, *R. brasiliensis* (BRUNER 1904) (Figura 1) é encontrada na região neotropical e se destaca por sua ampla distribuição em todas as regiões do Brasil (ASSIS-PUJOL, 1998). Particularmente no Nordeste, a espécie foi registrada nos estados do Rio Grande do Norte, Paraíba, Pernambuco (ASSIS-PUJOL, 1998) e na Bahia (LORETO *et al.,* 2008a). Em Pernambuco, essa espécie foi registrada nos municípios de Araripina, Bonito, Gravatá, Recife, Sanharó, Caruaru (ASSIS-PUJOL, 1998), Serra Talhada, Bezerros, Surubim, Toritama, Jaboatão dos Guararapes, Itamaracá e Cabo (LORETO *et al.,* 2008a), evidenciando sua distribuição em todas as mesorregiões do estado.

Segundo ASSIS-PUJOL (1998) os indivíduos machos e fêmeas de *R. brasiliensis* são morfologicamente semelhantes, no entanto a fêmea é consideravelmente mais robusta e de tamanho mediano, enquanto os machos

possuem tamanho pequeno. Além disso, possuem asas bem desenvolvidas que confere alta mobilidade e capacidade de dispersão (CARBONELL, 1988).

Figura 1. Exemplar adulto macho do gafanhoto *Rhammatocerus brasiliensis*

Fonte: Celso A. Ferreira-Neto

De acordo com Carbonell C. S. (comunicação pessoal) a espécie *R. brasiliensis* possui dois morfotipos distintos, denominados típico e atípico. O morfotipo típico é caracterizado por uma faixa branca na superfície dorsal do pronoto que divide duas áreas laterais escuras, separando a carena lateral no eixo ântero-posterior. Enquanto que o morfotipo atípico apresenta pronoto de cor uniforme sem a faixa branca, a carena lateral encontra-se separada apenas na região posterior.

2.2 CROMOSSOMO B

Os cromossomos supernumerários ou cromossomos B são elementos adicionais dispensáveis do cariótipo e constituem um dos polimorfismos mais difundidos em várias espécies de eucariontes (CAMACHO, 2005). O cromossomo B foi descrito pela primeira vez por Wilson em 1907 como cromossomo supernumerário nas espécies *Metapodius terminalis*, *M. femoratus* e *M. granulosus* (Hemiptera) e nomeado como cromossomo B por Randolph em 1928. Os Bs são caracterizados por não obedecerem às leis de herança mendeliana e pela ausência de recombinação com os cromossomos do complemento A (CAMACHO *et al.,* 2000).

A evolução do cromossomo B ocorre através do acúmulo de mutações exclusivas em suas sequências de DNA repetitivo, que podem variar em relação ao tipo de repetição e números de cópia, resultante de *crossing-over* desigual e ausência de recombinação (CAMACHO *et al.,* 2000; CAMACHO, 2005). O sequenciamento de diferentes DNAs repetitivos presentes no cromossomo B de várias espécies demonstraram que algumas destas sequências são específicas para o B, sendo as demais compartilhadas pelos cromossomos A (VALENTE *et al.,* 2014; RAMOS *et al.,* 2016; HOUBEN, 2017).

Os cromossomos Bs podem se originar de forma intraespecífica, a partir de um cromossomo autossômico ou sexual da espécie hospedeira, ou de forma interespecífica, a partir do genoma de espécies próximas (CAMACHO, 2005). No primeiro caso, o B seria produto da evolução do cariótipo da espécie, originado por fragmentos cêntricos oriundos de fusões dos cromossomos A ou de amplificação de um desses fragmentos (CAMACHO *et al.,* 2000). Os exemplos mais comuns se referem a fissão de cromossomos autossômicos como observado nas espécies

Drosophila subsilvestris (GUTKNECHT *et al.,* 1995) e na vespa *Nasonia vitripennis* (MCALLISTER e WERREN 1997). No segundo caso, em que a origem é interespecífica, ocorre a transposição ou hibridização interespecífica, como observado no peixe *Poecilia formosa*, espécie híbrida entre *P. mexicana e P latipinna* (SCHARTL *et al.,* 1995) e em insetos da ordem Hymenoptera, como *N. vitripennis* (MCALLISTER e WERREN 1997).

Quanto ao tamanho, os cromossomos Bs são classificados em quatro categorias: 1) cromossomos B médios, com tamanho semelhante ao X; 2) B pequeno, com tamanho semelhante ao menor par do complemento; 3) B minúsculo ou diminuto, menor do que qualquer elemento do cariótipo normal, e 4) B isocromossomo, que na meiose pode formar um anel. Os cromossomos Bs podem ser estáveis mitótica ou meioticamente, caso apresentem o mesmo número de Bs em todas as células dos indivíduos que os possuem ou instáveis quando seu número varia em diferentes células (JONES e REES 1982; HOUBEN *et al.,* 2013).

Os cromossomos B podem ser encontrados em diferentes números entre os indivíduos de uma população e quando presentes em número elevado podem causar efeitos deletérios, devido ao desequilíbrio na dosagem gênica (JONES *et al.,* 2008; CARLSON, 2009; HOUBEN *et al.,*, 2013). Muitos deles apresentam comportamento egoísta durante a transmissão, o que leva a uma vantagem para a invasão de novas populações e manutenção cromossômica do B em populações naturais (JONES *et al.,* 2008).

A ocorrência do cromossomo B é praticamente universal e, de acordo com D'Ambrosio *et al.* (2017), esse cromossomo ocorre no cariótipo de 2.828 espécies que correspondem a cerca de 15% dos eucariotos analisados (plantas, fungos, insetos, parasitas helmintos, crustáceos, peixes, anfíbios, répteis, aves e mamíferos)

e 11,9% dos insetos da ordem Orthoptera (JONES e REES 1982; CAMACHO 2005; PALESTIS *et al.,* 2010; JONES, 2017). A aparente ausência desse elemento em alguns táxons pode estar relacionada à carência de estudos populacionais e a baixa frequência deste cromossomo nas populações analisadas (CAMACHO, 2005).

A frequência de cromossomos B em diferentes populações naturais depende de um conjunto de fatores que podem atuar simultaneamente, sendo eles: históricos, que correspondem ao número de gerações desde o surgimento do B; de transmissão, que se refere a intensidade de acumulação do B (mecanismos de *drive*) entre populações; seletivos, relacionados a tolerância ecológica dos indivíduos com B; e aleatórios, como as flutuações genéticas sofridas por populações de tamanho finito (CAMACHO *et al.,* 2000).

Os estudos de cromossomos B classicamente abordam a ocorrência, prevalência, número (PASTORI e BIDAU 1994; BIDAU *et al.,* 2004; LORETO *et al.,* 2008b; MACHADO *et al.,* 2014; BERNARDINO *et al.,* 2017) e a localização de marcadores cromossômicos obtidos através de bandeamentos (BAKKALI *et al.,* 1999; LORETO *et al.,* 2008b; BUENO *et al.,* 2013; JETYBAYEV *et al.,* 2018). A partir dos anos 2000, os trabalhos em gafanhotos e outros organismos têm utilizado o mapeamento de sequências repetitivas (DNA ribossomal, DNA satélite, histona H3, DNAsn U2, elementos transponíveis) e análises moleculares (análises populacionais e de composição molecular) visando revelar a origem (BIDAU *et al.,* 2004; TERUEL *et al.,* 2010; BUENO *et al.,* 2013; MILANI *et al.,* 2018), comportamento (MILANI *et al.,* 2017), composição e função de sequências presentes no cromossomo B (OLIVEIRA *et al.,* 2011; RUIZ-RUANO *et al.,* 2016) e aspectos referentes a evolução dessas sequências (BUENO *et al.,* 2013; PALACIOS-GIMENEZ *et al.,* 2014; JETYBAYEV *et al.,* 2018).

As análises de composição do cromossomo B, visando indícios da origem, em insetos foram realizadas principalmente em espécies de gafanhotos (*Locusta migratoria, Eyprepocnemis plorans, Schistocerca rubiginosa, Xyleus discoideus angulatus* e *Abracris flavolineata*) (CABRERO *et al.,* 2003a; TERUEL *et al.,* 2010; BUENO *et al.,* 2013; MILANI *et al.,* 2018). Contudo, alguns trabalhos também propuseram a origem do cromossomo B em outros insetos, como os besouros *Dichotomius sericeus* (AMORIM *et al.,* 2016) e *Coprophanaeus cyanescens* (OLIVEIRA *et al.,* 2012), a mosca *Drosophila melanogaster* (HANLON *et al.,* 2018) e a abelha *Partamona helleri* (MARTINS *et al.,* 2013).

A composição das sequências presentes no cromossomo B microdissectado do peixe *Astatotilapia latifasciata* foi analisado com base no sequenciamento de nova geração e revelou que este cromossomo contém milhares de sequências que foram duplicadas de quase todos os cromossomos dessa espécie (VALENTE *et al.,* 2014). De acordo com Valente *et al.,* (2014), embora a maioria dos genes nos cromossomos B sejam fragmentados, alguns estão em grande parte intactos, como exemplo, genes envolvidos no controle do ciclo celular, na organização dos microtúbulos, estruturas do cinetócoro, recombinação e progressão no ciclo celular.

Estudos em peixes também evidenciaram genes e/ou sequências relacionas a recombinação e reparo do DNA (WALNE *et al.,* 2013), determinação do sexo (YOSHIDA *et al.,* 2011), envolvidos na não disjunção e preferencial segregação na meiose I em fêmeas (CLARK *et al.,* 2017), composição de elementos transponíveis (TEs) presentes no B e diferencial expressão de TEs entre indivíduos com e sem B (COAN and MARTINS 2018) e sequências de DNA transcricionalmente ativas (RAMOS *et al.,* 2016), as quais podem beneficiar o hospedeiro do cromossomo B.

2.2.1 Cromossomos B em gafanhotos

No reino animal, segundo Palestis *et al*. (2010), 76% das espécies com ocorrência de polimorfismo para cromossomo B são insetos, principalmente ortópteros, cujo percentual corresponde a 52,28% (D'AMBROSIO *et al.,* 2017). Estes cromossomos são os polimorfismos mais comuns dentro de Orthoptera, com cerca de 191 espécies portadoras de cromossomos B que constitui 11,9% das espécies estudadas (PALESTIS *et al.,* 2010). Entre os ortópteros, a ocorrência de cromossomos B é maior em espécies da superfamília Acridoidea (HEWITT, 1979; JONES e REES 1982; CAMACHO, 2005; PALESTIS *et al.,* 2010). A família Acrididae possui o maior percentual de espécies portadoras de cromossomos B (17,1%) e é considerada um *hotspot* para presença desse cromossomo (PALESTIS *et al.,* 2010).

Em algumas espécies de gafanhoto foram observados mais de um B, como em *Aeropus sibiricus* com quatro Bs, todos acrocêntricos (JETYBAYEV *et al.,* 2018) e *Eumastusia koebelei koebelei* com dois cromossomos Bs acrocêntricos, apresentando condensação similar ou diferencial entre eles, dependendo das células analisadas (ANJOS *et al.,* 2016). A ocorrência de indivíduos com mais de um B provavelmente é resultante da não disjunção das cromátides irmãs desse elemento durante a meiose, levando ao acúmulo de cromossomos B nas células (CAMACHO, 2005).

Entre as espécies de gafanhoto, a melhor estudada até o momento quanto ao polimorfismo de cromossomo B foi *Eyprepocnemis plorans*, na qual foram descritos mais de 50 tipos distintos de Bs (CAMACHO *et al.,* 2003). A técnica de bandeamento C contribuiu para a identificação dos diferentes Bs em *E. plorans*, revelando 14 tipos de B, considerando o padrão de localização dos blocos de HC,

com Bs quase inteiramente heterocromáticos até aqueles com pouca HC (HENRIQUES-GIL *et al.,* 1984). A distribuição geográfica de diferentes variantes de cromossomo B em *E. plorans* tem sido amplamente estudada na Espanha e a presença de cromossomos B foi relatada em quase todas as populações naturais analisadas (CAMACHO *et al.,* 1980; HENRIQUES-GIL *et al.,* 1984; HENRIQUES-GIL e ARANA 1990). Além disso a espécie também foi estudada em nove populações do Marrocos, tendo apresentado seis variantes para o cromossomo B (BAKKALI *et al.,* 1999).

A frequência de cromossomos B em acridídeos foi descrita em várias espécies, em *Melanoplus femur-rubrum* com 10% (NUR, 1977), *E. plorans* de 10 a 15% (HENRIQUES-GIL *et al.,* 1984), *Xyleus discoideus* de 0 a 25% (BERNARDINO *et al.,* 2017) e *Rhammatocerus brasiliensis* de 6,7 a 18,8% (LORETO *et al.,* 2008b). Em populações onde a frequência do cromossomo B é igual ou maior que 25% foi sugerido que o B tenha origem recente (ARAÚJO *et al.,* 2001), e nos casos em que a frequência foi relativamente baixa, foi proposto que o B estava estável ou em processo de extinção (RIERA *et al.,* 2004).

Embora a presença de cromossomos B nos gafanhotos seja comum e bem relatada, as análises sobre a composição do B em populações neotropicais são restritas as espécies *R. brasiliensis* (LORETO *et al.,* 2008b; OLIVEIRA *et al.,* 2011), *Xyleus discoideus angulatus* (LORETO *et al.,* 2008b; BERNARDINO *et al.,* 2017), *Dichroplus pratensis* (BIDAU *et al.,* 2004), *Abracris flavolineata* (BUENO *et al.,* 2013). Em populações neárticas, o maior número de estudos tem sido realizado em *E. plorans* (LÓPEZ-LEÓN *et al.,* 1994; BAKKALI *et al.,* 2001; CABRERO *et al.,* 2003b), *Locusta migratoria* (TERUEL *et al.,* 2010) e espécies do gênero *Podisma* (BUGROV *et al.,* 2007).

O mapeamento de sequências repetitivas de DNA em espécies de gafanhotos com B compreendem principalmente as sequências de DNA ribossomal DNAr 45S e 5S, Histona H3, snDNA U2, DNA satélite e microssatélite, e tem permitido o maior entendimento sobre a origem e evolução deste cromossomo (LORETO *et al.,* 2008b; TERUEL *et al.,* 2010; BUENO *et al.,* 2013; MILANI e CABRAL-DE-MELLO 2014; RUIZ-RUANO *et al.,* 2016; MILANI *et al.,* 2018). O uso de sequências repetitivas para verificar a origem de cromossomo B através da FISH foi feito, em *E. plorans* cujas análises sugeriram a origem intraespecífica da variante B_{24} a partir do X, com base em sequências de DNA ribossomal e DNA satélite (180 pb) (TERUEL *et al.,* 2009) e em *Locusta migratoria* que apresentou a localização de histona H3 apenas no par 8 e no B (TERUEL *et al.,* 2010).

Em gafanhotos neotropicais, alguns estudos abordaram a origem do B a partir do mapeamento de sequências repetitivas, como em *X. d. angulatus* cujo mapeamento de DNAr 45S e Histona H4 em indivíduos de nove populações, revelou que não havia ocorrência desses sítios no cromossomo B, sugerindo que o B não se originou a partir dos cromossomos portadores dos sítios DNAr 45S (par cromossômico 3 e o X) e da histona H4 (pares cromossômicos 2, 4 e X) (LORETO *et al.,* 2008b; MACHADO *et al.,* 2014).

Em *A. flavolineata*, foram mapeadas sequências de DNAr 5S e 45S, histona H3, sequências teloméricas, fração de C0t-1 do DNA e DNAsn U1 e U2 que sugeriu a origem do B a partir do par 1, cujos os sítios de DNAsn U2 foram localizados e compartilhados com o B (BUENO *et al.,* 2013). Também foi observado em *A. flavolineata* a distribuição de 16 unidades repetitivas de microssatélites, com repetições mono, di, tri e tetranucleotídicas, que podem estar envolvidas na

evolução do B, embora não tenha sido observada relação específica com o complemento A (MILANI e CABRAL-DE-MELLO 2014).

Entre os acridídeos neotropicais a espécie *R. brasiliensis* (*2n* = 23, X0) (♂), mostrou ampla distribuição de cromossomo B e variação de número devido a presença deste polimorfismo, em indivíduos com um B (*2n* = 24, X0 + B) e dois Bs (*2n* = 25,X0 + 2Bs) (LORETO *et al.,* 2008a; OLIVEIRA *et al.,* 2011). A ocorrência do cromossomo B em *R. brasiliensis* foi observada em sete populações do estado de Pernambuco (Itamaracá, Gravatá, Bezerros, Bonito, Toritama, Serra Talhada, Surubim), com ocorrência de indivíduos com dois B em duas dessas populações (LORETO *et al.,* 2008a, b; OLIVEIRA *et al.,* 2011).

No cromossomo B de *R. brasiliensis* também foi observado um bloco de heterocromatina constitutiva pericentromérico e riqueza da HC em pares de bases GC (LORETO *et al.,* 2008b), indicando assim que esse cromossomo possui acúmulo de DNA repetitivo que pode ser resultante da ausência de recombinação entre o complemento A com o cromossomo B.

O mapeamento cromossômico de genes de DNAr 5S na espécie *R. brasiliensis* foi realizado por Loreto *et al.* (2008b) e os sítios foram observados nos pares L2, L3, M5, S11 e no cromossomo B. No entanto, Oliveira *et al.,* (2011) observaram resultados distintos quanto ao número de *cluster* de DNAr 5S, os quais foram localizados em todos os cromossomos do complemento, exceto S11, e no cromossomo B, evidenciando assim a dispersão desses sítios no genoma da espécie. Além disso, foi observado ampla dispersão das sequências de histona H3 nos cromossomos de *R. brasiliensis* (OLIVEIRA *et al.,* 2011), esse resultado difere do observado em gafanhotos já que na maioria das espécies encontra-se conservada em um único par autossômico (CABRERO *et al.,* 2009). A espécie *R.*

brasiliensis também foi mapeada quanto ao gene U1 e apresentou localização nos bivalentes quatro, seis e sete (ANJOS *et al.,* 2014). A tabela 1 mostra os dados das análises cromossômicas realizadas em *R. brasiliensis* até o momento.

Tabela 1: Análises cromossômicas realizadas em *Rhammatocerus brasiliensis*.

Análises citogenéticas	Resultados	Referências
Convencional	*2n = 23, X0/24, XX; **2n = 24, X0/25, XX +1B ***2n = 25, X0/26, XX +2Bs	Loreto *et al.,* (2008b)
Bandeamento C	Blocos de HC Pericentroméricos em todos os cromossomos	Loreto *et al.,* (2008b)
Tríplice coloração	Blocos Pericentroméricos ricos em GC^+	Loreto *et al.,* (2008b)
FISH (DNAr 18S)	Sítios pericentroméricos nos pares M4, M6 e S9	Loreto *et al.,* (2008b)
FISH (DNAr 5S)	Sítios pericentroméricos nos pares L2, L3, M5 e S11	Loreto *et al.,* (2008b)
	Sítios pericentroméricos em todos os pares (exceto S11) e no B	Oliveira *et al.,* (2011)
FISH (Histona H3)	Sítios pericentoméricos em todos os pares (exceto S11) e no B Apenas no M7 em 1 indivíduo	Oliveira *et al.,* (2011)
FISH (snRNA U1)	Sítios intersticiais no bivalente M4 e M7; sítio centromêrico e subdistal no M6	Anjos *et al.,* (2014)

**2n* = 23, X0 em 608 indivíduos; ***2n* = 24, X0 +1B em 75 indivíduos e ****2n* = 25, X0 +2B em 1 indivíduo.

3 Wide dispersion of B chromosomes in *Rhammatocerus brasiliensis* (Orthoptera, Acrididae)

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ABSTRACT

The grasshopper *Rhammatocerus brasiliensis* shows polymorphism of B chromosomes, even though the magnitude of B-chromosome occurrence and the factors that may contribute to their dispersion in the species have been unknown thus far. The present study analyzed the occurrence and dispersion of B chromosomes in *R. brasiliensis* individuals from 21 populations widely distributed in the Brazilian Northeast. The genetic connectivity between ten populations was verified through analysis of ISSR markers from 200 individuals. Of the 21 populations, 19 presented individuals with one B chromosome, three with two B, and one with three B chromosomes. The B chromosome has medium size and constitutive heterochromatin (CH) located in the pericentromeric region. A variant B chromosome, displaying size similar to that of chromosome X, gap and CH additionally located in the terminal region, was observed in three populations. The frequency of B chromosomes in different populations varied from 0 % to 18.8 %, mean 8.5 %. The wide distribution of the B chromosome is likely a consequence of the positive gene flow among the analyzed populations. B-chromosome occurrence in populations of *R. brasiliensis* possibly follows the population genetic structure of the species and, owing to the existence of a variant, its origin may not be recent.

Keywords: Grasshopper, Supernumerary chromosome, Chromosome polymorphism, ISSR, Genetic connectivity.

INTRODUCTION

B chromosomes, also known as supernumerary chromosomes, are extra elements of the karyotype. These chromosomes do not obey the Mendelian laws of inheritance and do not present homology with the other chromosomes of the A complement (Camacho *et al.,* 2000). Moreover, they generally differ by the accumulation of repetitive DNA sequences, such as satellite DNA (RUIZ-RUANO *et al.,* 2017; MILANI *et al.,* 2017a), ribosomal DNA and histone H3 (OLIVEIRA *et al.,* 2011), U2 small nuclear RNA (BUENO *et al.,* 2013), and transposable elements (PALACIOS-GIMENEZ *et al.,* 2014). Although repetitive sequences are common in the composition of B chromosomes, also genes conferring resistance to antibiotics and pathogens have been identified (COLEMAN *et al.,* 2009), besides others that participate in the control of the cell cycle (VALENTE *et al.,* 2014; RAMOS *et al.,* 2016).

The occurrence of B chromosomes is almost universal, being found in approximately 15% of the analyzed eukaryotes (CAMACHO, 2005). Among the animals, the insects of the order Orthoptera stand out for the greater frequency of B chromosomes, being hotspots in the superfamilies Acridoidea, Grylloidea, Pyrgomorphoidea and Tetrigoidea (PALESTIS *et al.,* 2010). In Acridoidea, the Acrididae family considered a hotspot representing 17.1 % (PALESTIS *et al.,* 2010) where *Locusta migratoria* (LINNAEUS, 1758) and *Eyprepocnemis plorans* (CHARPENTIER, 1825) are used as models to better understand the occurrence, origin and evolution of the B chromosomes (BUENO *et al.,* 2013).

When the frequency of B chromosomes in a population is equal to or higher than 25 %, it is suggested that their origin is recent (ARAÚJO *et al.,* 2001). Contrarily, in populations where the frequency is relatively low (below 25 %), the B chromosome

is considered stable or in process of elimination (RIERA *et al.,* 2004). According to some authors (CAMACHO *et al.,* 1997, 2015; ZURITA *et al.,* 1998), B chromosomes present four main life-cycle stages: 1) Invasion, where a B chromosome arrives or emerges in a natural population and its frequency rises rapidly within only a few tens of generations; 2) Resistance, when a B chromosome remains in the host genome through drive mechanisms; 3) Near neutrality, when the frequency of B chromosomes varies randomly by genetic drift, or decreases due to negative selection in individuals with increased number of B chromosomes (this constitutes the longest stage, which can last for thousands of generations); and 4) Regeneration, where the B chromosome depends on the accumulation of mutations for the emergence of novel variants that may be able to remain in the genome and substitute the former, neutralized B chromosome, reinitiating the B chromosome cycle and prolonging its evolutionary life.

For Neotropical grasshoppers, particularly those occurring in Brazil, most works performed so far have focused on the analysis of the origin and composition of B chromosomes through the mapping of different repetitive sequences, as observed for the species *Abracris flavolineata* (*2n* = 24, X0 + B and *2n* = 25, X0 + 2Bs) (BUENO *et al.,* 2013; MILANI e CABRAL-DE-MELLO 2014; PALACIOS-GIMENEZ *et al.,* 2014; MENEZES-DE-CARVALHO *et al.,* 2015; MILANI *et al.,* 2017a,b) and *Xyleus discoideus angulatus* (*2n* = 24, X0 + B and *2n* = 25, X0 + 2Bs) (LORETO *et al.,* 2008a; MACHADO *et al.,* 2014; BERNARDINO *et al.,* 2017). For *Rammatocerus brasiliensis*, also the frequency of B chromosomes has been analyzed in eight populations from Pernambuco and three from Bahia, states of the Brazilian Northeast, showing *2n* = 24, X0 + B and *2n* = 25, X0 + 2Bs (LORETO *et al.,* 2008a, b; OLIVEIRA *et al.,* 2011). Regarding Neotropical species, the distribution of B

chromosomes in natural grasshopper populations has not been analyzed under consideration of the genetic structure of the populations; such studies have only been performed in Palearctic grasshoppers (KELLER *et al.,* 2013; MANRIQUE-POYATO *et al.,* 2013a, b, 2015). This kind of approach allows estimating the gene flow among individuals within and between populations, which may be a parameter directly related to the dispersion or isolation of B chromosomes (MANRIQUE-POYATO *et al.,* 2015).

R. brasiliensis is widely distributed in the Northeast of Brazil, with records in the states of Rio Grande do Norte, Paraíba, Pernambuco (ASSIS-PUJOL, 1998) and Bahia (LORETO *et al.,* 2008a). In Pernambuco, the species is found across the entire state, having been reported in eleven localities (CARBONELL 1995; ASSIS-PUJOL, 1998; LORETO *et al.,* 2008a). Because of its wide distribution (CARBONELL, 1988), *R. brasiliensis* is an interesting species for understanding the life-cycle stages of B chromosomes and how they may disperse. In this study, the following hypotheses were tested: 1) B chromosomes are dispersed among *R. brasiliensis* populations according to gene flow existing among them; 2) Based on the relatively low frequency of B chromosomes in *R. brasiliensis*, as verified in different populations by Loreto *et al.,* (2008b), it is expected that the B chromosome is currently in the near-neutrality stage.

This way, we analyzed the occurrence, frequency and distribution of B chromosomes in *R. brasiliensis* individuals from 21 populations from the Brazilian Northeast, of which ten were also analyzed regarding population genetic structure. In addition, the position of the constitutive heterochromatin (CH) was established in the B chromosomes of the species.

MATERIAL AND METHODS

Sampling

Specimens of *R. brasiliensis* (Bruner 1904) were collected in 21 populations from the Brazilian Northeast, of which 13 were located in the state of Pernambuco (PE), one in Sergipe (SE), one in Alagoas (AL), one in Paraíba (PB), one in Ceará (CE), one in Piauí (PI), and three in Bahia (BA) (Figure 1). Of these populations, 11 have been previously sampled and analyzed with regard to the occurrence and prevalence of B chromosomes by Loreto *et al.,* (2008a) and Oliveira *et al.,* (2011).

The grasshoppers were collected with the help of an entomological net and transported to the Laboratory of Insect Biodiversity and Genetics (LBGI) – Institute of Biological Sciences at the University of Pernambuco. The collections were permitted by IBAMA/SISBIO with the license number 16278-1.

Conventional staining and C-banding

The karyotypes of 590 adult male specimens of *R. brasiliensis* were analyzed by conventional staining. First, the classic technique of testicular follicle squashing was performed, consisting in the maceration of the gonads in one drop of 45 % acetic acid. Subsequently, the slides were subjected to heat of 65 °C for approximately 3 minutes for fixation of the chromosome preparation onto the slide, then stained with 5 % Giemsa. The C-banding technique was performed according to Sumner (1972), with duration of exposure to the basic solution ($Ba(OH)_2$) altered to 2 min, in individuals carrying B chromosomes from the populations of VIT and LC (Pernambuco – PE).

Analysis of occurrence and frequency of B chromosomes and statistical test

The occurrence, frequency and distribution of B chromosomes were analyzed in a total of 1,274 specimens. Of these, 394 were individuals from ten populations being analyzed for the first time (GOI–PE, LC–PE, SAL–PE, BUI–PE, OUR–PE, ARA–SE, MAC–AL, JP–PE, JN–PE and PIC–PI) and 196 were individuals from four populations with samplings being expanded (ITA–PE, VIT–PE, BEZ–PE and ST–PE). The data for the remaining specimens were obtained from the literature (LORETO *et al.,* 2008b; OLIVEIRA *et al.,* 2011), thus gathered to provide a wider sampling for this species.

Using the software OpenEpi 3.01, a statistical significance test was carried out based on Fisher's exact test in order to verify whether the frequency of B chromosomes differs significantly among the populations. Further, using the same software, analysis of confidence interval (CI) was performed to estimate the maximum and minimum number that the frequency of B chromosomes may reach in an analysis with a population size of 1,000,000 specimens.

DNA extraction and ISSR marker amplification

The genomic DNA of *R. brasiliensis* specimens was extracted from the leg muscle according to protocol described by Sambrook and Russell (2001). Subsequently, the DNA was purified through enzymatic digestion with RNAse (10 mg/mL) for 1 h at 37°C. Next, the concentration and quality of the extracted DNA were verified via electrophoresis in 1% agarose gel by comparison with DNA from phage Lambda DNA/Hind III marker (Fermentas Life Sciences).

Polymerase chain reaction (PCR) amplification was performed from a total volume of 10 µL containing 5 ng of DNA, 1X PCR buffer, 5 mM of $MgCl_2$, 0.2 mM of dNTPs (2.5 mM), 1 pmol of primer (0.2 mM) and 1 U Taq polymerase (Invitrogen). The reactions were carried out in a thermocycler (Biosystems) as follows: Standard program at 94 °C for 4 min; 35 cycles at 94 °C for 30 s; annealing step (with temperature varying for each primer) for 45 s; extension at 72 °C for 2 min; and final extension at 72 °C for 7 min. The PCR products were visualized in 1.8 % agarose gel using a transilluminator (Gel Logic/Carestream MI SE).

A total of 49 ISSR primers (Biotechnology Laboratory, University of British Columbia, Canada) were tested in one *R. brasiliensis* individual, with 33 presenting higher number of amplified DNA fragments with good resolution. Selection of the most informative primers was accomplished by applying the 33 pre-selected primers to a sample of nine specimens from three populations (PIC–PI, JN–CE and JP–PB). Based on the genotyping, the data were grouped into a binary matrix of presence and absence. The analysis of the most informative primers was carried out according to the parameters number of total, monomorphic and polymorphic DNA fragments; and polymorphism indices PIC -Polymorphism Information Content (ROLDÁN-RUIZ *et al.,* 2000), MI - Marker Index (PREVOST AND WILKINSON 1999) and RP - Resolving Power (VARSHNEY *et al.,* 2007). Further, the Pearson correlation was calculated among the PIC, MI and RP indices. The seven most informative primers (807, 835, 844, 845, 846, 857, 866 – supplementary Table 1) were applied to 200 individuals from ten populations, with 20 obtained from each population, thus representing the wide distribution of the species in the Northeast of Brazil (Figure 1; Table 1).

Reproducibility test

The reproducibility of the ISSR markers was verified through analyses based on two independent PCR reactions for all seven primers that were applied to all individuals of the studied populations. Non-reproducible *loci* were excluded from the genetic analysis.

Genetic diversity and population structure

The genetic diversity index (GD), mean expected heterozygosity (H_E), Wright's fixation index (F_{ST}) and molecular variance (AMOVA) were calculated with the software Arlequin 3.5 (EXCOFFIER AND LISCHER 2010). The number of migrants (Nm) and coefficient of genetic differentiation (G_{ST}) were calculated in the software Popgen 1.31 (YEH *et al.,* 1999). Further, the level of isolation by distance (IBD) was analyzed from the correlation between genetic and geographical distances (Mantel test – SOKAL AND ROHLF 1995) using the software Arlequin 3.5 (EXCOFFIER AND LISCHER 2010), based on 10,000 permutations. At last, the population model most adequate to the data was established with the software Hickory 1.1 (HOLSINGER *et al.,* 2002) considering four possible models: i) Full model, where the values of population differentiation (theta, θ, an analog of Wright's parameter F_{ST}) and inbreeding (f, an analog of Fis) differ from zero; ii) model f=0, which assumes absence of inbreeding within the populations; iii) model theta=0, based on the absence of population differentiation; and iv) the free model, where the values are chosen randomly, independent of a-priori information. The choice of the most suitable model was based on the parameters deviance information criterion (DIC), an analog of the Akaike information criterion (AIC) in the selection via Bayesian models,

and Dbar, a measure of the level of adjustment with which the model adapts to the analyzed data (the lower the value of both, the better).

Number of genetic clusters

The genetic clusters were analyzed with the software Structure 2.3.1 (PRITCHARD *et al.,* 2000) using the Bayesian algorithm, where the K value was identified and analyzed to determine the level of genetic mixture among the populations. For each value of K (from 1 to 10), ten independent rounds were performed with 600,000 replications and burn-in of 60,000 interactions. Finally, we used the website Structure Harvester (EARL, 2012), which implements the method of Evanno (EVANNO *et al.,* 2005), to estimate the number of genetic groups (K) which best adjust the data.

RESULTS

Cytogenetic data

The karyotype *2n* = 23, X0, described for the species *R. brasiliensis*, was observed in 1.162 specimens. All chromosomes of the complement are acrocentric and were categorized, by size, into large (L1–L3), medium (M4– M8) and small pairs (S9–S11). In addition, *2n* = 24, X0 + B was seen in 107 individuals, with the B chromosome being acrocentric and having medium size (Figures 2 a, b, d) in all populations exhibiting this polymorphism (ITA–PE, GOI–PE, VIT–PE, LC–PE, GRA–PE, SAL–PE, BON–PE, BUI–PE, SUR–PE, BEZ–PE, TOR–PE, ST–PE, OUR–PE, MAC–AL, JN–CE, PIC–PI, SOB–BA, RC–BA, and ITAB–BA); *2n* = 25, X0 + 2Bs (Figure 2b) was observed in four specimens from ITA–PE, BEZ–PE, and OUR–PE; and *2n*=26, X0 + 3Bs in one individual from ITA–PE (Figure 2c, insert) (Table 2). Two distinct B chromosomes were observed: one verified in 19 of the 21 analyzed

populations, of medium size and smaller than X; and another, here denoted as variant, identified in the populations from ITA, BEZ and LC, with size similar to that of X and presence of a gap in the long arm (Figure 2 c). In specimens with a single chromosome B, association with the X chromosome was observed (Figure 2a), whereas in the case of two B chromosomes, association occurred with autosomes instead (Figure 2b).

The presence of B chromosomes was verified in almost all populations, with the exception of ARA–SE and JP–PB. Among populations with B chromosomes ITAB–BA presented the highest frequency (18.8 %), and SAL–PE the lowest (2.32 %). The values for total frequency of males with B chromosomes in the populations varied from 0 % to 18.8 %, with a mean of 8.5 % (Table 2). Significant difference existed in this regard between some populations (p values > 0.05) identified by Fisher's pairwise exact test, namely: ITA–JP, 0.032; GRA–SUR, 0.031; GRA–ST, 0.012; GRA–OUR, 0.045; GRA–MAC, 0.008; GRA–JP, 0.007; GRA–JN, 0.031; GRA–PIC, 0.045; and BEZ–JP, 0.038 (Table 3).

The C-banding procedure revealed positive C bands in the pericentromeric region of all chromosomes of the A complement, as well as in the B chromosomes (Figure 2d). An additional block was observed in the terminal region of the variant B chromosome (Figure 2e).

Genetic diversity and population structure data

The seven ISSR primers used in the ten analyzed populations generated a total of 95 reproducible fragments, which varied in size between 300 and 2,500 base pairs (bp). Among the 95 analyzed loci, 71.05 % were polymorphic, with the populations OUR–PE and ARA–SE presenting the highest and lowest percentages of

polymorphism (90.5 % and 13.6 %), respectively (Table 4). In addition, the mean value for expected heterozygosity (H_E) in the populations was 0.311, with OUR–PE and MAC–AL presenting the highest and lowest values, corresponding to 0.36 and 0.28, respectively (Table 4).

The analysis of population models indicated the full model and f=0 as the most adequate according to the parameters DIC and Dbar (SPIEGELHALTER *et al.,* 2002). However, the model f=0, which assumes absence of inbreeding within the populations, is the most probable, owing to the gene flow evidenced by other analyses (F_{ST}, AMOVA) and the dispersion capacity of *R. brasiliensis* (Table 5).

Molecular variance (AMOVA) reached 15.32 % among the populations and 84.68% within them. Moreover, low genetic differentiation and high gene flow were observed upon analysis of the fixation index, F_{ST} = 0.15; the coefficient of genetic differentiation, G_{ST} = 0.17; and the number of migrants, Nm = 2.3869. Most of the pairwise F_{ST} values were low, though significant values were obtained for the populations JN–CE and MAC–AL, ST–PE and ITA–PE, ST–PE and BUI–PE, and JN–CE and ST–PE (Table 6). The Mantel test revealed lack of correlation between the genetic and geographical distances (r: 0.023121; P: 0.828000), which indicates the absence of IBD.

The genetic clustering analysis performed with the software Structure presented K=2 as highest value, according to the method described by Evanno *et al.,* (2005) (Figure 3), with two genetic groups being observed in all analyzed populations (Figure 4).

DISCUSSION

B chromosomes in *R. brasiliensis*

The polymorphism of the B chromosome found in *R. brasiliensis* is well represented in Acrididae grasshoppers (PALESTIS *et al.,* 2010). The broad distribution of *R. brasiliensis* individuals carrying B chromosomes suggests that this polymorphism is widespread among the populations, apparently there is a relation between the higher Bs frequency and the geographical distribution, for example in GRA-PE, BEZ-PE, SUR-PE and VIT-PE. This tendency is likely associated positive gene flow among those populations (better discussed below). Moreover, studies in other grasshopper species such as *Eyprepocnemis plorans* (CAMACHO *et al.,* 1980), *Trimerotropis pallidipennis* (CONFALONIERI, 1992) and *Dichroplus elongatus* (REMIS, 1989) reported increase in the frequency of recombination and chiasmata in host species, resulting in an advantageous increase in genetic variability (CAMACHO *et al.,* 1980, 2002; RIERA *et al.,* 2004), which may be one of the reasons for the wide distribution of B chromosomes in *R. brasiliensis*.

The frequency of B chromosomes in *R. brasiliensis* was considered high (above 11 %) and low (below 5 %). However, all populations have values below 25 %, which is indicative of the phase in which the B chromosome is in the life cycle (CAMACHO *et al.,* 1997; ARAÚJO *et al.,* 2001). Given the values of the observed frequencies, the B of *R. brasiliensis* populations is probably in the stage of near neutrality of its life cycle. This is the longest phase, with successive generations being necessary to disperse the B chromosome, which has strictly vertical transmission. These frequencies may arise from stability, considering that these chromosomes tend to reach a balanced frequency over the years due to the efforts of the host genome to extinguish the B chromosome (CAMACHO *et al.,* 1997; RIERA *et al.,* 2004).

The non-detection of B chromosomes in JP–PB and ARA–SE may be related either to elimination of the polymorphism or to low frequency in these populations as

a manner of defense of the host genome, which attempts to get rid of parasite chromosomes (CABRERO *et al.,* 2017). Most of the analyzed populations did not show significant difference with regard to the occurrence and frequency of B chromosomes. This may be associated to low frequency of B chromosomes in several populations and/or relatively low number of sampled populations lacking these chromosomes. The sampling number of these groups was possibly insufficient to detect the occurrence of B chromosomes, a fact that was corroborated by the confidence interval calculation, which indicated maximum frequency of 8.2 % in JP–PB and 26 % in ARA–SE. In *R. brasiliensis*, the different frequencies may be associated with the sum of several other factors, such as the number of generations since the origination or emergence of the B chromosome; population differences in the accumulation of the B chromosome, in case this polymorphism brings an advantage to the host in a population; and action of genetic drift (in particular for ARA–SE, based onits low genetic diversity; Table 4), which may be eliminating individuals carrying B chromosomes.

The occurrence of *R. brasiliensis* individuals with two B chromosomes in the populations of BEZ–PE, OUR–PE and ITA–PE, as well as three B chromosomes in ITA–PE, is also observed in some Acrididae, for instance *Aeropus sibiricus* with four acrocentric B chromosomes (JETYBAYEV *et al.,* 2018) and *Eumastusia koebelei koebelei*, with two acrocentric B chromosomes (ANJOS *et al.,* 2016). The presence of more than one B chromosome in *R. brasiliensis* is probably due to the non-disjunction of its sister chromatids during meiosis, as suggested by Camacho (2005). Some mechanisms may be acting in this phenomenon, such as a control region of non-disjunction located in the B chromosome itself (in the terminal or pericentromeric region), as already observed in rye (Endo *et al.,* 2008; Banaei-Moghaddam *et al.,*

2012). Moreover, B chromatids non-disjunction could involve B specific products (proteins or ncRNAs) that could associate with the centromeric regions and retard the separation during anaphase, resulting in both B chromatids end up in the gamete (BENETTA *et al.,* 2019). Various B-chromosome transcripts related to genes encoding proteins or pseudo-genes in rye (BANAEI-MOGHADDAM *et al.,* 2013), fish (VALENTE *et al.,* 2014; RAMOS *et al.,* 2016), fly (BAUERLY *et al.,* 2014) and cervids (MAKUNIN *et al.,* 2016) provides the basis to hypothesize about the involvement of protein-coding genes or pseudogenes in non-disjunction control. The constitutive heterochromatin (CH) may also act on the cohesion of the sister chromatids of the B chromosome, leading to nondisjunction (BANAEI-MOGHADDAM *et al.,* 2012). However, the CH of B chromosomes from *R. brasiliensis* observed in this study, as well as by Loreto *et al.* (2008a) and Milani *et al.* (2018), is restricted to the pericentromeric and/or terminal region, indicating that other of the mechanisms listed above may be acting on the nondisjunction of the B chromosomes.

The small amount of CH restricted to the pericentromeric region of the B chromosomes from *R. brasiliensis* is different from that observed in other grasshopper species that possess B chromosomes with CH amplification rich in satellite DNA (LORETO *et al.,* 2008A; BERNARDINO *et al.,* 2017; MILANI *et al.,* 2018). However, the hypothesis cannot be discarded that other repetitive DNA segments may be dispersed in euchromatic regions, such as transposable elements (TEs), which can be present in both observed types of B chromosomes (common and variant). TEs may have accumulated in the B chromosome and promoted the amplification of sequences (MARQUES *et al.,* 2018), contributing to the generation of the variant B chromosome.

B chromosomes can be differentiated with the C-banding technique based on the patterns of CH distribution, as observed in different types of B chromosomes in the grasshopper *E. plorans* (HENRIQUES-GIL *et al.,* 1984). In addition, the terminal region of the variant B chromosome found in the population from LC–PE was also C-positive, which further contributes to the characterization of the variant B chromosome. This variant is probably undergoing the regeneration stage of the cycle, where it has accumulated modifications in order to escape neutralization by the host genome (HENRIQUES-GIL AND ARANA 1990; CAMACHO *et al.,* 1997). This way, the hypothesis of non-recent origin of B chromosomes is reinforced, since successive generations are necessary for the B chromosome to be nearly neutralized, accumulate modifications and scatter in the populations (HENRIQUES-GIL AND ARANA 1990; CAMACHO *et al.,* 1997).

Genetic connectivity between populations of *R. brasiliensis*

As previously observed in other organisms (TAYLOR *et al.,* 2011; PINHEIRO *et al.,* 2012; IZZATULLAYEVA *et al.,* 2014), the ISSR marker was polymorphic in *R. brasiliensis*, with 71% of the loci presenting variation in the ten analyzed populations. The expected heterozygosity (H_E) presented variation between 0.285 in MAC–AL and 0.363 OUR–PE. These values are similar to those described for other insects, such as the grasshoppers *Pezotettix giornae* (GAUFFRE *et al.,* 2015) and *E. plorans* (MANRIQUE-POYATO *et al.,* 2013a); the bee *Apis mellifera meda* (RAHIMI *et al.,* 2016); as well as the low value in the beetle *Canthon staigi* (FERREIRA-NETO *et al.,* 2017) and the high value in *Lucanus cervus* (SNEGIN, 2014). Although it has already been evinced that, in grasshoppers, the presence of B chromosomes may contribute to the increase in genetic variability, this relationship was not observed for *R.*

brasiliensis, in which some populations present low H_E, such as ST–PE (0.291) and JN–CE (0.290). Moreover, the total expected heterozygosity (H_T= 0.298) was greater than the subpopulation heterozygosity (H_S= 0.270), indicating that there may be a deficit of heterozygotes.

The data generated in this study suggest that the occurrence of B chromosomes in *R. brasiliensis* is related to its population genetic structure, given the wide dispersion of the B chromosome verified in the karyotyped individuals, positive gene flow (Nm = 2.3869), and low genetic differentiation F_{ST} (0.15) and G_{ST} (0.17) among the analyzed populations. Owing to the migratory behavior of *R. brasiliensis* (CARBONELL, 1988), this is an expected pattern, considering that the probable means of dispersion of the B chromosome in different geographic ranges, according to Camacho *et al.* (2015), would be through gene flow between hosts and individuals from populations that do not have the B chromosome. The Mantel test suggesteda lack of correlation between genetic and geographical distance, even between populations more than 700 km apart (ITA–PE and JN–CE). This may be contributing for the dispersion of the B chromosomes, as their transmission is strictly vertical (MUNOZ *et al.,* 1998). Pairwise F_{ST} indicated an overall low genetic differentiation; however, some populations presented F_{ST} with significantly high values (JN–CE and MAC–AL, JN–CE and ST–PE, ST–PE and ITA–PE, ST–PE and BUI–PE; Table 6).

The data from molecular variance (AMOVA), which was greater within the populations (84.68%), when associated to Structure data, corroborate the low indices of genetic differentiation, where the presence of a mixture of two genetic profiles is related to migratory habit, wide mobility and flight capacity of *R. brasiliensis* (CARBONELL 1988, 1995). The native grasses, for instance *Trachypogon sp.* (Poaceae), constitute the preferred food of grasshoppers (SILVA *et al.,* 2006).

Therefore, the large offer of grasses in the localities sampled in this study and the absence of effective geographic barriers between the populations are fundamental factors for the wide dispersion of the analyzed specimens. The feeding preference of *R. brasiliensis*is strongly related toits mandibular structure, of graminivorous type, also present in other Gomphocerinae such as *Achurum carinatum*, *Dichromorpha viridis* and *Orphulella pelidna* (SMITH AND CAPINERA 2005). For other grasshopper species, for instance *Mioscirtus wagneri* and *Ramburiella hispanica*, a positive relationship was also observed between the distribution of plants used as food and the genetic connectivity between populations (ORTEGO *et al.,* 2010, 2015).

Although the populations of Pernambuco and other states encounter different altitudes (range 0 to 999 m) as well as potential geographic barriers (Borborema Plateau, Chapada do Araripe and São Francisco river), *R. brasiliensis* individuals seem to be capable of overcoming these obstacles and maintaining a positive gene flow among the analyzed populations. This way, no correlation was observed between the occurrence of B chromosomes and the distribution of *R. brasiliensis* in different landscapes. For this species, the altitude of the analyzed populations did not seem to influence the frequency of B chromosomes either. This observation differs from that of Manrique-Poyato *et al.* (2015), who stated that individuals with B chromosomes have low tolerance to high-altitude environments, as a result of delay in the meiosis due to the presence of B chromosomes that may negatively select the host (HEWITT AND EAST 1978; HARVEY AND HEWITT 1979).

Based on literature evidence that B chromosomes are only transmitted vertically, it is suggested that, for the species *R. brasiliensis*, the broad distribution of B chromosomes indeed arises from the gene flow extant among the analyzed populations. The migratory habit coupled to the high flying capacity of *R. brasiliensis*,

besides the wide offer of food resources in the sampled localities, are factors that certainly contribute for the genetic connectivity of its populations, and consequently for the broad dispersion of the B chromosome. A variant B chromosome, with CH blocks in the pericentromeric and terminal regions, was detected and is probably currently in the regeneration stage of the cycle. The presence of one such variant B chromosome reinforces the hypothesis of the non-recent origin of the B chromosome, considering that successive generations are necessary for such a chromosome to be nearly neutralized, accumulate modifications and be dispersed among distinct populations.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest .

AUTHOR CONTRIBUTIONS

RCM, ASM, MFR, conceived and designed the experiments; ASM, APF, conducted the experiments; ASM, GASC, RCM, analyzed the data and wrote the paper; RCM, VL, critically revised the manuscript. All authors read and approved the final version.

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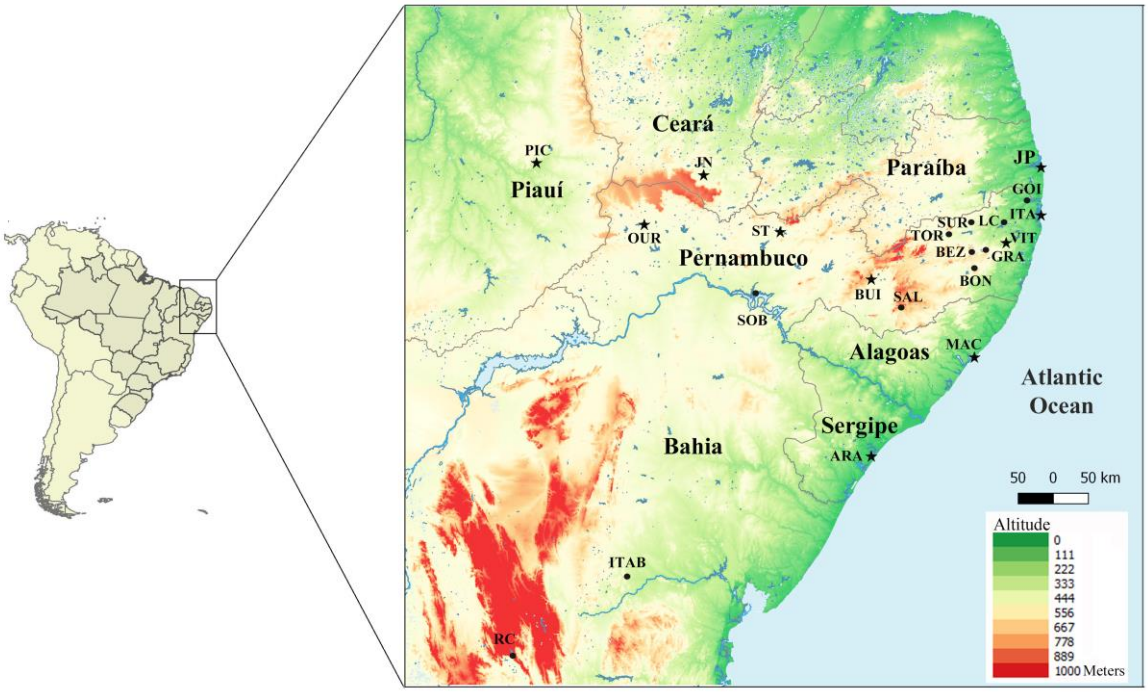

Figure 1. Partial map of the Brazilian Northeast indicating the sampling locations of *Rhammatocerus brasiliensis* populations. Aracaju (ARA) – SE; Maceió (MAC) – AL; João Pessoa (JP) – PB; Itamaracá (ITA) – PE; Vitória de Santo Antão (VIT) – PE; Goiana (GOI) – PE; Gravatá (GRA) – PE; Bonito (BON) – PE; Toritama (TOR) – PE; Saloá (SAL) – PE; Buíque (BUI) – PE; Lagoa do Carro (LC) – PE; Surubim (SUR) – PE; Bezerros (BEZ) – PE; Serra Talhada (ST) – PE; Ouricuri (OUR) – PE; Juazeiro do Norte (JN) – CE; Picos (PIC) – PI; Sobradinho (SOB) – BA; Itaberaba (ITAB) – BA; and Rio de Contas (RC) – BA. Circles indicate populations with karyotyped individuals. Stars indicate populations with karyotyped individuals also analyzed for population genetics.

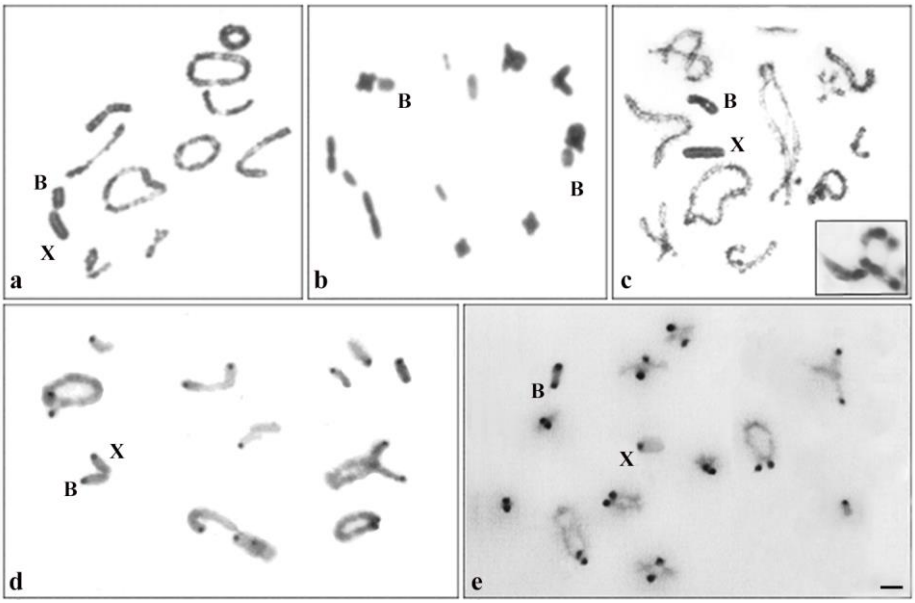

Figure 2. Meiotic cells of the species Rhammatocerus brasiliensis conventionally stained (a, b, c) and C-banded (d, e). a, c, d, e) Diplotene stage with one B chromosome. b)Diakinesis with two B chromosomes. c) Observe the variant B and the insect with three variant B chromosomes. Bar = 5 µm.

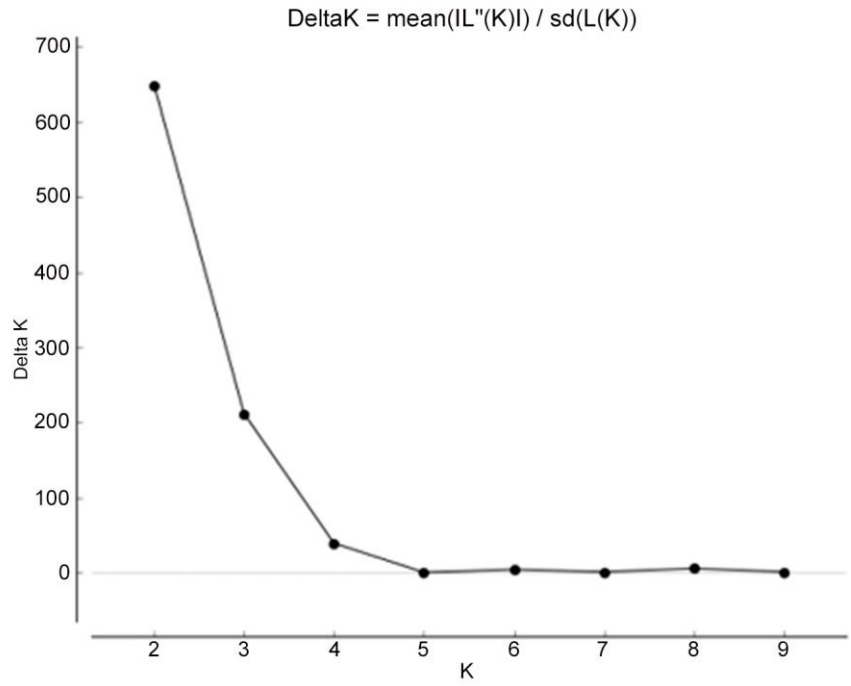

Figure 3. Delta K values according to the method of Evanno *et al.,* (2005). Note the highest peak of K = 2.

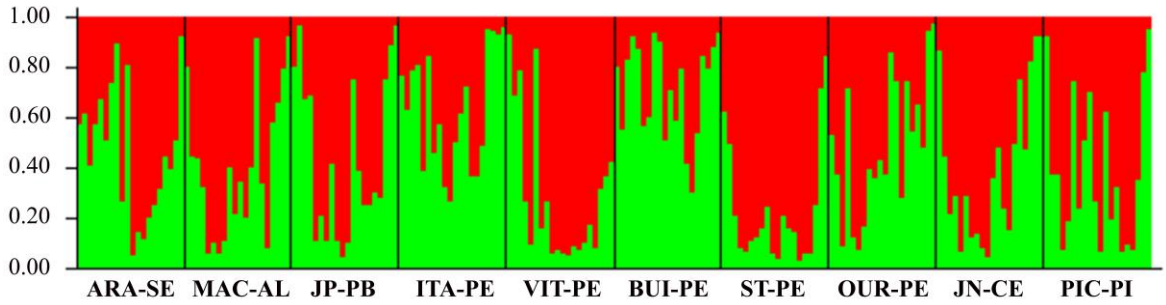

Figure 4. Ancestry of *Rhammatocerus brasiliensis* specimens traced by the software Structure version 2.3.1, using seven ISSR primers and 95 polymorphic bands. Each vertical bar corresponds to one of the 200 analyzed individuals and its population of origin. The bar length is proportional to parentage values inferred in each group for each individual.

Table 1 - State, mesoregion, altitude and geographic coordinate for the sampled populations of *Rhammatocerus brasiliensis* in the Northeast of Brazil.

State/ Mesoregion	Locality	Altitude	Coordinate geographic	Predominant biome / sampled area
Pernambuco/ Metropolitana do Recife	ITA - PE	3m	07°45'0"S; 34°05'10"W	Atlantic forest - Mangrove/open area
	GOI - PE	13m	07º33'38"S; 35º00'9"W	Atlantic forest - Restinga/open área
Pernambuco/ Zona da Mata	VIT - PE	162m	08º07'35"S; 35º18'27"W	Atlantic forest/open area
	LC - PE	128m	07º50'56"S; 35º19'14"W	Atlantic forest/open area
Pernambuco/ Agreste	GRA - PE	447m	08º12'04"S; 35º33'53"W	Atlantic forest - Brejo de altitude/open area
	SAL - PE	745m	08º58'33"S; 35º41'15"W	Caatinga/open área
	BON - PE	443m	08º28'13"S; 35º43'43"W	Atlantic forest - Brejo de Altitude/open area
	BUI - PE	798m	08º37'23"S; 37º09'21"W	Atlantic forest - Brejo de Altitude/open area
	SUR - PE	394m	07º59'31"S; 38º17'54"W	Caatinga/open área
	BEZ - PE	470m	08º14'00"S; 35º47'49"W	Atlantic forest - Brejo de Altitude/open area
	TOR - PE	349m	08°00'24"S; 36°03'24"W	Caatinga/open área
Pernambuco/ Sertão	ST - PE	444m	07º49'59"S; 35º45'17"W	Caatinga/open area
	OUR - PE	451m	07º52'57"S; 40º04'54"W	Caatinga/open área
Aracaju/ Leste Sergipano	ARA - SE	4m	10º59'07"S; 37º04'24"W	Atlantic forest - Restinga/open area
Alagoas/ Leste Alagoano	MAC - AL	4m	09º39'59"S; 35º44'06"W	Atlantic forest - Restinga/ open area
Paraíba/ Mata Paraibana	JP - PB	37m	07º06'54"S; 34º51'47"W	Atlantic forest - Restinga/ open area
Ceará/ Sul Cearense	JN - CE	377m	07º12'47"S; 39º18'55"W	Caatinga/ open area
Piauí/ Sudeste Piauiense	PIC - PI	206m	07º04'37"S; 41º28'01"W	Caatinga/ open area
Bahia/ Vale São Franciscano	SOB - BA	0	09º27'19"S; 40º49'24"W	Caatinga/ open area
Bahia/ Centro-Sul	RC - BA	999m	13º34'44"S; 41º48'41"W	Caatinga/ open area
Bahia/ Centro-Norte	ITAB - BA	265m	12º31'39"S; 40º18'25"W	Caatinga/ open area

Table 2 - Occurrence, distribution, frequency and confidence interval of B chromosomes in the analyzed populations of *Rhammatocerus brasiliensis* in the Northeast of Brazil.

Locality	Individuals 1B	Individuals > 1B	Total	Prevalence (%)	Confidence Interval 95%	Number of individuals analyzed / reference
ITA - PE	8	2	73	13,69	7,2 - 23%	27/ Loreto *et al.*, 2008b e 46/ Present study
GOI - PE	4		36	11,11	3,6 - 25%	Present study
VIT - PE	11		101	10,89	5,8 - 18%	57/ Oliveira *et al.*, 2011 e 44/ Present study
LC - PE	4		27	14,8	4,9 - 32%	Present study
GRA - PE	15		84	17,85	10 - 28%	Loreto *et al.*, 2008b
SAL - PE	1		43	2,32	0,12 - 11%	Present study
BON - PE	8		64	12,5	6 - 22%	Loreto *et al.*, 2008b
BUI - PE	1		27	3,7	0,18 – 16%	Present study
SUR - PE	11		147	7,48	4 - 13%	Oliveira *et al.*, 2011
BEZ - PE	15	2	142	11,97	7,4 - 18%	88/ Loreto *et al.*, 2008b e 54/ Present study
TOR - PE	6		66	9,1	3,8 - 18%	Loreto *et al.*, 2008b
ST - PE	9		144	6,25	3,1 - 11%	12/ Loreto *et al.*, 2008b; 80/ Oliveira *et al.*, 2011 e 52/ Present study
OUR - PE	1	1	46	4,35	0,7 - 14%	Present study
ARA - SE	0		10	-	0 - 26%	Present study
MAC - AL	2		63	3,17	0,5 - 10%	Present study
JP - PB	0		35	-	0 - 8,2%	Present study
JN - CE	3		61	4,92	1,3 - 13%	Present study
PIC - PI	2		46	4,35	0,7 - 14%	Present study
SOB - BA	2		30	6,7	1,1 - 20%	Loreto *et al.*, 2008b
RC - BA	2		13	15,4	2,7 - 42%	Loreto *et al.*, 2008b

ITAB - BA	3		16	18,8	5 - 43%	Loreto *et al.*, 2008b
Total	107	5	1274			

Table 3 - Statistical significance by Fisher's exact test between populations of *Rhammatocerus brasiliensis* analyzed for the presence of B chromosomes. Values of $p < 0.05$ are in boldface.

Locality	ITA	GOI	VIT	LC	GRA	SAL	BON	BUI	SUR	BEZ	TOR	ST	OUR	ARA	MAC	JP	JN	PIC	SOB	RC	ITAB
ITA-PE	-	0.961	0.739	0.633	0.625	0.077	0.999	0.287	0.220	0.871	0.562	0.119	0.173	0.510	0.057	**0.032**	0.152	0.173	0.517	0.999	0.851
GOI-PE		-	0.999	0.965	0.523	0.258	0.999	0.559	0.673	0.999	0.993	0.494	0.458	0.721	0.251	0.121	0.457	0.458	0.855	0.999	0.733
VIT-PE			-	0.908	0.252	0.154	0.936	0.462	0.480	0.962	0.921	0.285	0.326	0.671	0.128	0.065	0.306	0.326	0.778	0.908	0.586
LC-PE				-	0.315	0.658	0.762	0.350	0.999	0.765	0.999	0.999	0.947	0.999	0.694	0.371	0.977	0.947	0.999	0.783	0.519
GRA-PE					-	0.017	0.511	0.113	**0.031**	0.303	0.191	**0.012**	**0.045**	0.318	**0.008**	**0.007**	**0.031**	**0.045**	0.231	0.999	0.999
SAL-PE						-	0.121	0.999	0.396	0.093	0.314	0.569	0.999	0.999	0.999	0.999	0.900	0.999	0.732	0.262	0.113
BON-PE							-	0.375	0.360	0.999	0.731	0.217	0.255	0.587	0.100	0.051	0.236	0.255	0.642	0.999	0.764
BUI-PE								-	0.831	0.353	0.682	0.999	0.999	0.999	0.999	0.871	0.999	0.999	0.999	0.484	0.274
SUR-PE									-	0.275	0.876	0.855	0.722	0.945	0.387	0.177	0.739	0.722	0.999	0.570	0.287
BEZ-PE										-	0.720	0.138	0.217	0.587	0.067	**0.038**	0.188	0.217	0.633	0.985	0.656
TOR-PE											-	0.633	0.570	0.831	0.304	0.143	0.573	0.570	0.999	0.783	0.482
ST-PE												-	0.952	0.999	0.590	0.268	0.995	0.952	0.999	0.454	0.206
OUR-PE													-	0.999	0.999	0.638	0.999	0.999	0.999	0.415	0.206
ARA-SE														-	0.999	0	0.999	0.999	0.999	0.616	0.430
MAC-AL															-	0.821	0.969	0.999	0.775	0.266	0.107
JP-PB																-	0.503	0.638	0.418	0.138	0.053
JN-CE																	-	0.999	0.999	0.419	0.200
PIC-PI																		-	0.999	0.415	0.206
SOB-BA																			-	0.700	0.441
RC-BA																				-	0.999
ITAB-BA																					-

Table 4 - Genetic diversity (GD) and mean heterozygosity (H_E) estimated for the analyzed populations of *Rhammatocerus brasiliensis*.

Locality	**H_E**	**DG**
ARA - SE	0,312	13,6
MAC - AL	0,285	68,4
JP - PB	0,320	83,1
ITA - PE	0,297	74,7
VIT - PE	0,329	50,5
BUI - PE	0,324	87,3
ST - PE	0,291	81,0
OUR - PE	0,363	90,5
JN - CE	0,290	82,1
PIC - PI	0,298	78,9
	0,311	71,0

Table 5 - Parameters estimated by the software Hickory for the model $f = 0$.

Parameteres	**Mean**	**S.D.**	**2.5%**	**97.5%**
Theta-I	0.223	0.016	0.193	0.258
Theta-II	0.104	0.006	0.092	0.117
Theta-III	0.087	0.003	0.080	0.094
Theta-Y	0.132	0.018	0.101	0.172
Rho	0.591	0.041	0.512	0.673
Hs [ARA]	0.257	0.005	0.245	0.268
Hs [MAC]	0.257	0.005	0.246	0.268
Hs [JP]	0.270	0.005	0.259	0.281
Hs [ITA]	0.259	0.005	0.247	0.270
Hs [VIT]	0.293	0.005	0.282	0.304
Hs [BUI]	0.262	0.006	0.250	0.273
Hs [ST]	0.279	0.005	0.268	0.291
Hs [OUR]	0.290	0.005	0.279	0.301
Hs [JN]	0.279	0.005	0.269	0.290
Hs [PIC]	0.259	0.005	0.248	0.269
Hs	0.270	0.002	0.267	0.274
H_T	0.298	0.002	0.294	0.302
$G_{ST}B$	0.092	0.004	0.084	0.100

Table 6 - Mantel test based on pairwise F_{ST} in ten analyzed populations of *R. brasiliensis*.

Locality	ARA - SE	MAC - AL	JP - PB	ITA - PE	VIT - PE	BUI - PE	ST - PE	OUR - PE	PIC - PI	JN - CE
ARA - SE	-	208 km	488 km	437 km	374 km	260 km	360 km	475 km	488 km	652 km
MAC - AL	0,113	-	292 km	217 km	165 km	196 km	342 km	523 km	480 km	695 km
JP - PB	0,074	0,112	-	070 km	112 km	300 km	388 km	600 km	497 km	726 km
ITA - PE	0,184	0,183	0,129	-	057 km	269 km	381 km	589 km	499 km	729 km
VIT - PE	0,145	0,128	0,123	0,183	-	216 km	334 km	533 km	458 km	694 km
BUI - PE	0,166	0,161	0,077	0,113	0,203	-	142 km	326 km	284 km	500 km
ST - PE	0,156	0,147	0,171	0,240	0,124	0,226	-	197 km	138 km	363 km
OUR - PE	0,154	0,165	0,119	0,154	0,113	0,146	0,159	-	124 km	163 km
JN - CE	0,179	0,229	0,139	0,130	0,182	0,162	0,224	0,162	-	232 km
PIC -PI	0,128	0,178	0,098	0,127	0,134	0,164	0,191	0,116	0,098	-

4 Inferências sobre o papel do elemento *Gypsy* na ampla dispersão da histona H3 no genoma do gafanhoto *Rhammatocerus brasiliensis*

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RESUMO

O mapeamento dos sítios de histonas H3 e H4 tem sido realizado em gafanhotos e, em sua maioria, a localização dos sítios é conservada. No gafanhoto *Rhammatocerus brasiliensis* foi observada a localização pericentromérica dos sítios de H3, os quais estão dispersos em quase todos os cromossomos do complemento e nos cromossomos B. Este trabalho objetivou entender a dispersão dos *clusters* de histona H3 e o possível papel de elementos transponíveis no mecanismo de dispersão dessa sequência. Um total de 88 indivíduos da espécie *R. brasiliensis* foram analisados por hibridização *in situ* fluorescente (FISH) com sondas de histona H3, oriundos de 13 populações do Nordeste brasileiro. Indivíduos de algumas dessas populações também tiveram o retroelemento *Gypsy* mapeado. Dois padrões de localização da H3 foram observados, conservado e disperso, esse último de forma moderada ou ampla, a qual também mostrou variação. Os sítios de H3 foram localizados na região pericentromérica nos cromossomos do complemento A e no B, e o par cromossômico M7 foi identificado como portador do sítio ancestral. Os dados do mapeamento do elemento *Gypsy*, em 10 indivíduos também mapeados para H3, mostraram que esse elemento encontra-se amplamente disperso e localizado na região pericentromérica. Na maioria dos indivíduos analisados, o *Gypsy* apresentou localização similar ao observado para H3, diferindo apenas quando a H3 encontra-se restrita ao M7. A ampla dispersão da H3 em *R. brasiliensis* possivelmente resulta de recombinações ectópicas promovidas pela ação do retroelemento *Gypsy*, o qual foi caracterizado de um *cluster* com compartilhamento de histona. Apresentou ainda três domínios conservados, sugerindo que trata-se de um elemento autônomo. O *Gypsy* pode ter se refugiado nas regiões pericentroméricas, ricas em heterocromatina constitutiva, consideradas zonas genômicas com baixa densidade gênica, e por estar presente e disperso nas populações estudadas, possivelmente se estabeleceu e pode estar exercendo alguma função estrutural no centrômero da espécie hospedeira.

Palavras-chaves: Gafanhoto, DNA repetitivo, FISH, Histona, Retrotransposon.

INTRODUÇÃO

O DNA repetitivo corresponde às sequências de DNA que se repetem centenas a milhares de vezes no genoma e compreende a maior porção do genoma nuclear dos eucariontes (BISCOTTI *et al*., 2015), com percentual que varia em gafanhotos entre 60% em *Pyrgomorpha conica* (RUIZ-RUANO *et al*., 2018) a 64% em *Locusta migratoria* (WANG *et al*., 2014). O mesmo pode ser encontrado repetido em tandem, representado por microssatélites, minissatélites, DNA satélites e famílias multigênicas ou disperso no genoma, como os elementos de transposição (LÓPEZ-FLORES E GARRIDO-RAMOS 2012).

O DNA repetitivo tem sido amplamente estudado por apresentar grande importância na organização e função nos genomas dos eucariontes (UGARKOVIC *et al.,* 2005; PALOMEQUE E LORITE, 2008; LOWER *et al.,* 2018). Algumas sequências repetitivas são bastante conservadas entre as espécies e outras são variáveis, mostrando diferenças entre espécies estreitamente relacionadas (BISCOTTI *et al.,* 2015). Nas espécies de eucariontes superiores, as sequências de H3 e H4 são altamente conservadas, mesmo em espécies distantes de animais e plantas (NEI E ROONEY 2005). Em contraste, as sequências de DNA satélite, apresentam ampla variabilidade estrutural e grande quantidade de repetições nos genomas (LÓPEZ-FLORES E GARRIDO-RAMOS 2012). Diante disso, a comparação sobre a natureza e localização das sequências repetitivas entre espécies ou indivíduos podem fornecer informações sobre sua evolução e amplificação, já que algumas sequências são extremamente bem conservadas entre as espécies e outras são mais variáveis (BISCOTTI *et al.,* 2015).

Nos gafanhotos, o mapeamento de DNA repetitivos foi realizado em um maior número de espécies utilizando sondas de DNAs ribossomal e de histonas H3 e H4 (CABRERO *et al*., 2003; LORETO *et al.,* 2008a,b; CABRERO *et al*., 2009; TERUEL *et al*., 2010; CABRAL-DE-MELLO *et al.,* 2011a, B; OLIVEIRA *et al*., 2011; BUENO *et al*., 2013; PALACIOS-GIMENEZ *et al.,* 2013, 2015a; SILVA-NETO *et al.,* 2015; CASTILLO *et al.,* 2017). Em representantes da família Acrididae, foram descritos diferentes padrões de distribuição dessas sequências, com variação de um a nove sítios de DNAr 45S (CABRERO E CAMACHO 2003; LORETO *et al.,* 2008a) e presença de sítios de DNAr 5S apenas em um bivalente ou disperso em todos os pares cromossômicos do complemento (CABRAL-DE-MELLO *et al*. 2011a). Essas variações no número de sítios de DNAr 5S foram observadas nos acridídeos

Eyprepocnemis plorans (de dois a 20 sítios) (CABRERO *et al.,* 2003; CABRAL-DE-MELLO *et al.,* 2011a) e *Amblytropidia sp.* (de 13 a 17 sítios) (SILVA-NETO *et al*., 2015). As sequências de histonas, em geral, apresentam alta conservação relacionada à localização cromossômica e número de *clusters* nos genomas em comparação a sequências de DNAr (CABRERO *et al.,* 2009; CABRAL-DE-MELLO *et al.,* 2011B; SILVA-NETO *et al.,* 2015; CASTILLO *et al.,* 2017). Esta característica também é observada em representantes da família Acrididae, estando o *cluster* de histona H3 presente em apenas um par cromossômico (CABRERO *et al.,* 2009; SILVA-NETO *et al.,* 2015; CASTILLO *et al.,* 2017). No entanto, através do mapeamento por FISH, foi observada a dispersão desses sítios em quatro espécies da família Acrididae, *Abracris flavolineata* (BUENO *et al*., 2013), *Dichromatos lilloanus* (PALACIOS-GIMENEZ *et al.,* 2013) *Rhammatocerus brasiliensis* (OLIVEIRA *et al*., 2011) e *Ronderosia bergi* (PALACIOS-GIMENEZ *et al*., 2015a).

O mapeamento de diferentes sequências repetitivas realizado no cariótipo de *R. brasiliensis* evidenciou a localização e dispersão das famílias multigênicas DNA ribossomal 18S e 5S, e histona H3 (LORETO *et al.,* 2008a; OLIVEIRA *et al*., 2011). Os genes de DNAr 18S e 5S mapeados por Loreto *et al*. (2008b) em *R. brasiliensis* foram localizados na região pericentromérica nos pares M4, M6 e S9 para o DNA 18S e nos pares L2, L3, M5, S11 e no cromossomo B para o DNAr 5S, e deram subsídios para as primeiras inferências sobre a possível origem do polimorfismo para cromossomo B observado na espécie. Oliveira *et al*. (2011) analisaram indivíduos de duas populações de Pernambuco-Brasil e observaram ampla dispersão dos *clusters* de DNAr 5S, os quais foram localizados em todos os cromossomos do complemento, exceto S11, e no cromossomo B.

A dispersão e colocalização das sequências de H3 com o DNAr 5S foram observadas em *R. brasiliensis*, com presença de sítios em todos os cromossomos do complemento, exceto o S11 (OLIVEIRA *et al*., 2011) e se destaca por ser a mais ampla quando comparada a dispersão da histona H3 em outras três espécies de gafanhotos (BUENO *et al.,* 2013; PALACIOS-GIMENEZ *et al.,* 2013, 2015a). Considerando que as sequências de H3 são conservadas quanto á localização em gafanhotos (CABRERO *et al*., 2009; CABRAL-DE-MELLO *et al*., 2011a), tem sido sugerido que recombinações ectópicas estariam propiciando a dispersão dessas sequências, em decorrência da ação de elementos transponíveis. Alguns trabalhos sugerem a relação dos elementos de transposição com a dispersão das sequências

de histona H3, como exemplo o peixe *Rachycentron canadum* (COSTA *et al*., 2014), o grilo *Gryllus assimilis* (PALÁCIOS-GIMENEZ *et al*., 2015b) e o besouro *Euchroma gigantea* (XAVIER *et al.,* 2018).

Recentemente, Pucci *et al*. (2018) sugeriram que os elementos transponíveis podem estar envolvidos na dispersão de sequências pela transposição das mesmas no genoma de peixes do gênero *Characidium*, com base na associação de sequências de histona H1 e H4 ao elemento. Adicionalmente, a alta homologia entre sequências provenientes da inserção de elementos em novos locais pode promover recombinações ectópicas, favorecidas pelas diversas cópias do elemento nos cromossomos (CRAIG *et al*., 2002; MACKAY, 2007; SANTANA *et al.,* 2014; MAUMUS *et al*., 2015).

Com o objetivo de obter uma melhor compreensão sobre a amplitude da dispersão das sequências de histona H3 e dos mecanismos envolvidos para a sua ocorrência no genoma de *R. brasiliensis*, realizamos uma análise de mapeamento populacional da sequência de histona H3 na espécie *R. brasiliensis* em 13 populações distribuídas em seis estados do Nordeste brasileiro. Adicionalmente foi mapeado o retrotransposon *Gypsy* em alguns indivíduos que apresentaram padrões diferentes de distribuição da H3.

MATERIAL E MÉTODOS

Coleta e processamento do material

Indivíduos machos da espécie *Rhammatocerus brasiliensis* Bruner 1904 foram coletados no período de 2012 a 2018, com o auxílio de rede entomológica em 13 localidades distribuídas em seis estados do Nordeste brasileiro (Figura 1). As coletas foram realizadas de acordo com as leis brasileiras de proteção ambiental e com a licença do IBAMA/SISBIO número 16278-1, emitida no dia 06/06/2008.

Preparação das lâminas

As lâminas foram preparadas através de técnica clássica de esmagamento de folículos, que consistiu no maceramento das gônadas dos machos em uma gota de ácido acético a 45%. Posteriormente, as lâminas foram submetidas a uma temperatura de 55°C em uma chapa quente para fixação do material à lâmina, por 3 min. As lâminas foram analisadas ao microscópio óptico e as melhores lâminas

foram selecionadas e armazenadas a uma temperatura de -20°C para utilização na técnica de hibridização *in situ* fluorescente (FISH).

Extração de DNA, Sequenciamento e Seleção de elemento transponível

O DNA genômico de um indivíduo de *R. brasiliensis*, oriundo da população de Itamaracá-PE (ITA-PE), foi extraído de acordo com o protocolo descrito por Sambrook e Russel (2001). O DNA genômico desse indivíduo foi sequenciado através da plataforma Ilumina HiSeq 2000 de baixa cobertura.

As sequências obtidas foram clusterizadas no Repeat Explorer para análise de Elementos de Transposição (TEs). A abundância relativa foi analisada, bem como a identificação de *clusters*. Dentre eles foi selecionado o *cluster* 72 por ter revelado um grupamento de sequência gênica de histona H3 com um retrotransposon da superfamília *Gypsy*. Adicionalmente, foram realizadas as análises de remontagem no CAP3, identificação de ORFs pela ferramenta ORFfinder e caracterização de domínios pelo CDD do NCBI.

Obtenção das sondas e Hibridização *in situ* fluorescente (FISH)

As sondas de histona H3 e do TE da superfamília *Gypsy* foram obtidas por reação em cadeia da polimerase (PCR), a partir do DNA genômico de dois indivíduos da espécie *R. brasiliensis,* usando os *primers* universal de histona H3 ScaH3F (5’ ATG GCT CGT ACC AAG CAG ACV GC 3’) e ScaH3R (5’ ATA TCC TTR GGC ATR ATR GTG AC 3’) (Colgan *et al*. 1998) e do TE da família *Gypsy* GypF (5’-ACT TGT GGA AAT TGT GCT GGC-3’) e Gyp R (5’-TGC ATG CCG GAT ATT CGA TGA-3’). Para confirmar a presença dos produtos amplificados foi utilizada a técnica de eletroforese em gel de agarose a 1% e os produtos obtidos da PCR de H3 e Gypsy (com 330 pb e 744 pb, respectivamente) foram sequenciados e submetidos ao Blast (ALTSCHUL *et al*., 1990) para confirmar a similaridade com outras sequências depositadas previamente.

As sondas de histona H3 foram marcadas com digoxigenina (dig 11-dUTP - Roche) por PCR e detectadas com anti-digoxigenina-rodamina (Roche) e as do TE *Gypsy* foram marcadas com biotina (biotin 14-dATP - Invitrogen) através da reação de *nick translation* e detectadas por avidina-FITC (Invitrogen). A técnica de FISH foi realizada de acordo com o protocolo descrito por Pinkel *et al*. (1986) com modificações propostas por Cabral-de-Mello *et al*. (2010).

As sondas de histona H3 foram hibridizadas em células meióticas de 88 indivíduos oriundos de 13 populações, tendo 10 desses indivíduos também sido utilizados para o mapeamento do TE *Gypsy*. Indivíduos com cromossomo B de nove populações foram analisados, sendo seis de Pernambuco (um indivíduo de ITA-PE, ST-PE, OUR-PE, BUI-PE e dois de VIT-PE e LC-PE) e três de outros estados do Nordeste (um indivíduo de MAC-AL, JN-CE e PIC-PI). As imagens foram capturadas utilizando o fotomicroscópio de fluorescência LEICA DM 2500. O brilho e contraste das imagens foram otimizados e a construção das pranchas foram realizadas utilizando o Photoshop CS5.

RESULTADOS

A espécie *R. brasiliensis* apresentou o cariótipo 2n = 23, X0 (♂), com cromossomos acrocêntricos organizados em três grupos de acordo com o tamanho: grande (L1 - L3), médio (M4 - M8) e pequeno (S9 - S11) semelhante ao descrito por Loreto *et al*. (2008a). O cromossomo X apresentou tamanho médio e o cromossomo B foi um pouco menor que o X, enquanto o B variante possuiu tamanho similar ao X.

A análise do mapeamento de histona H3 em todos os indivíduos de *R. brasiliensis* mostrou dois padrões, diferindo quanto a localização conservada ou dispersa dos sítios de H3. O padrão conservado correspondeu a localização dos sítios de histona H3 apenas no bivalente autossômico M7 (Figura 2a) (em indivíduos oriundos de 10 populações), enquanto o padrão de dispersão dos sítios da H3 mostrou-se variável quanto ao nível de dispersão: (1) dispersão moderada, mostrou sítios em um ou dois cromossomos além do M7, como observado em indivíduos de LC-PE com marcação no M7 e no cromossomo B (Figura 2b) ou em indivíduos de VIT-PE com sítios nos bivalentes autossômicos L1, M4 e M7 (Figura 2c); e (2) dispersão ampla, quando apresentou marcação em quase todos os cromossomos do complemento, exceto S11 (em quatro populações), inclusive o B (em três populações) (Figura 2d) ou em todos os cromossomos do complemento (nove populações) e também com marcação no B (sete populações) (Figura 2e) (Figura 4a; Tabela 1).

Os cromossomos B foram observados em indivíduos oriundos de seis populações de Pernambuco e três de outros estados do Nordeste brasileiro. Todos os Bs observados apresentaram marcação para H3 (Figura 2b, d-e; 4a; Tabela 1).

O elemento *Gypsy*, aqui denominado *Rbra_Gypsy1*, possui os domínios conservados da transcriptase reversa, RNAseH e integrase, o que sugere que ele possivelmente seja autônomo. O mapeamento do elemento *Rbra_Gypsy1* em 10 indivíduos previamente analisados por histona H3 revelou um padrão de dispersão dessas sequências, com a localização das mesmas na região pericentromérica. Os sítios foram observados em quase todos os cromossomos do complemento, exceto S11 (população de ARA-SE) ou em todos os cromossomos do complemento (populações de MAC-AL e ARA-SE) (Figura 3b-d), como também no cromossomo B (VIT-PE e LC-PE) (Figura 3c-d). Nos indivíduos que apresentaram esses padrões de distribuição dos sítios de *Rbra_Gypsy1*, a marcação para H3 foi similar. O padrão de distribuição do *Rbra_Gypsy1* em quase todos os cromossomos do complemento, exceto S11 também foi observado em indivíduos (PIC-PI, GOI-PE e ST-PE) com marcação de H3 restrita ao par M7 (Figura 3a e 4b; Tabela 1).

DISCUSSÃO

O cariótipo da espécie *R. brasiliensis* observado neste estudo é considerado conservado quanto ao número e morfologia cromossômica, bem como sistema sexual. Alguns indivíduos apresentaram polimorfismo para cromossomo B, sendo portanto, similar aos cariótipos descritos por Loreto *et al*. (2008a) e Oliveira *et al*. (2011) para a espécie.

A presença dos sítios de histona H3 restrita ao par M7 no gafanhoto *R. brasiliensis* em 33 indivíduos, em dez das 13 populações aqui analisadas, bem como nos indivíduos que apresentaram a dispersão da H3, sugere que o sítio ancestral dessa sequência se encontra nesse bivalente. E que a sequência de H3 foi dispersada para outros cromossomos posteriormente. A localização da H3 em um par cromossômico de tamanho médio corrobora com o observado em outras espécies de acridídeos estudadas (CABRERO *et al.,* 2009; PALACIOS-GIMENEZ *et al.,* 2013; SILVA-NETO *et al.,* 2015; CASTILLO *et al.,* 2017; PINE *et al.,* 2017), que apresentam conservação da localização cromossômica de histonas, e que segundo Cabrero *et al*. (2009) essas sequências não sofreram alterações significativas desde a origem do grupo há cerca de 60 milhões de anos. A presença do sítio de histona ocorre com maior frequência em um dos pares autossômicos grandes (2 ou 3) ou médios (5 ou 8) nos acridídeos, e possivelmente sofre a ação de uma forte seleção purificadora atuando para manter a localização dessa sequência conservada no

genoma de espécies de gafanhotos (CABRERO *et al.,* 2009; PALACIOS-GIMENEZ *et al.,* 2013; SILVA-NETO *et al.,* 2015; CASTILLO *et al.,* 2017; PINE *et al.,* 2017).

O número de *cluters* dos genes de histona H3 parece ser altamente conservado não só em gafanhotos da família Acrididae, como também na família Proscopiidae, a segunda família mais antiga de gafanhotos (CABRAL-DE-MELLO *et al*., 2011c) e em outros grupos de insetos, como besouros do gênero *Dichotomius* (CABRAL-DE-MELLO *et al*., 2011b) e em *Lagria villosa* (GOLL *et al*., 2015), algumas espécies de mariposa (ŠÍCHOVÁ *et al*., 2013), percevejo (BARDELLA *et al*., 2016) e pulgão (MANDRIOLI E MANICARDI 2013) e cigarrinhas (ANJOS *et al*., 2018). Contudo, a ocorrência de número de *cluster* de histona H3 presente em mais de um bivalente cromossômico têm sido relatada em espécies de diferentes grupos de invertebrados, como no grilo *Gryllus assimilis* (PALACIOS-GIMENEZ *et al*., 2015), no pulgão *Diuraphis noxia* (NOVOTNÁ *et al*. 2011), no besouro *Euchroma gigantea* (XAVIER *et al*., 2018), escorpião *Tityus obscurus* (ALMEIDA *et al*., 2017), em espécies de moluscos (GARCÍA-SOUTO *et al*. 2015) e nos acridídeos *Dichromatos lilloanus, Abracris flavolineata* e *Ronderosia bergi* (BUENO *et al.,* 2013; PALACIOS-GIMENEZ *et al*., 2013, 2015). A dispersão dos sítios de H3 presente em todos os cromossomos de *R. brasiliensis* indica que a forte seleção purificadora proposta para explicar a conservação da localização da H3 em acridídeos (CABRERO *et al*., 2009), aparentemente não está atuando nesta espécie.

Os DNAs repetitivos presentes nas regiões heterocromáticas podem desempenhar um papel importante na dispersão de sequências, podendo promover variação na distribuição de sequências de histona entre indivíduos de *R. brasiliensis* de diferentes populações. Em *R. brasiliensis* também foi possível verificar abundância de DNAs repetitivos, de modo que é possível supor que a dinâmica dos elementos transponíveis (TEs) possa estar desencadeando rearranjos no genoma da espécie e assim promovendo a dispersão da histona, bem como de outros DNAs repetitivos mapeados em *R. brasiliensis* (LORETO *et al.,* 2008b; OLIVEIRA *et al.,* 2011; MILANI *et al*., 2018). Isto é plausível, visto que os genomas de gafanhoto possuem grande quantidade de sequências repetitivas, como observado em *Locusta migratoria*, que possui maior abundância para elementos repetitivos, que constituem cerca de 60% do genoma, sendo cerca de 24% transposons de DNA e 17% retrotransposons LINE (WANG *et al*., 2014).

A espécie *R. brasiliensis* apresentou variação no padrão de dispersão da histona H3, de moderada a ampla. Os dados observados indicam que a dispersão da sequência de H3 ocorreu a partir do bivalente M7 para os demais cromossomos, com a presença de sítios em mais um ou dois cromossomos até a dispersão completa (sítios em todos os cromossomos, inclusive o B). Como a histona H3 foi localizada na região pericentromérica dos cromossomos de *R. brasiliensis*, que é rica em outras sequências repetitivas, como: DNAr 18S e 5S (LORETO *et al.,* 2008b; OLIVEIRA *et al*., 2011), DNAsn U1 (ANJOS *et al.,* 2014), DNA satélite (MILANI *et al*., 2018) e o TE *Rbra_Gypsy1* mapeado neste estudo (Figura 4c), é possível supor a participação do *Rbra_Gypsy1* na dispersão dessas sequências, em especial dos sítios de H3, devido a sua localização semelhante na região pericentromérica em todos os cromossomos, exceto o S11, ou em todos os cromossomos, incluindo o B. A ação do TE *Rbra_Gypsy1*, que possivelmente é autônomo por possuir os domínios conservados da transcriptese reversa, RNAseH e integrase, pode estar resultando na dispersão das sequências de H3 através da promoção de regiões com alta homologia devido a sua inserção em novos locais no genoma *R. brasiliensis*, que consecutivamente deve ter levado a ocorrência de recombinações ectópicas entre os cromossomos (CRAIG *et al.,* 2002; MACKAY 2007; SANTANA *et al.,* 2014; MAUMUS *et al.,* 2015).

O padrão de localização e dispersão do TEs *Rbra_Gypsy1* em *R. brasiliensis* indica que esse elemento se refugiou nos centrômeros, que são considerados zonas de inserção genômicas “seguras”, tanto para o hospedeiro quanto para o TE, por possuir baixa densidade gênica evitando assim a ocorrência de mutação após a inserção (BIRCHLER E PRESTING 2012; SULTANA *et al.,* 2017). Este elemento *Rbra_Gypsy1* pode ainda estar relacionado a funções estruturais nos centrômeros dos cromossomos da espécie *R. brasiliensis*, o que justificaria sua localização preferencial pela região pericentromérica (PLOHL *et al.,* 2014).

O mapeamento cromossômico utilizando sondas de histona H3 em diferentes populações de *R. brasiliensis* contribuiu para um melhor entendimento da organizaçâo dos genes de histona H3 no genoma da espécie, bem como para verificar a amplitude da dispersão desses sítios. É interessante destacar que apesar das evidências de que o *Rbra_Gypsy1* favoreceu a dispersão da H3, não existem até o momento dados que permitam explicar o porquê da inserção das sequências de H3 e de *Rbra_Gypsy1* ocorrerem em todos os cromossomos, inclusive no B,

antes de se inserirem no S11, que é o par considerado ancestral do cromossomo B na espécie (MILANI *et al.,* 2018). De modo que outros estudos deverão ser feitos e poderão vir a descrever qual mecanismo pode está dificultando a inserção dessas sequências nesse par cromossômico.

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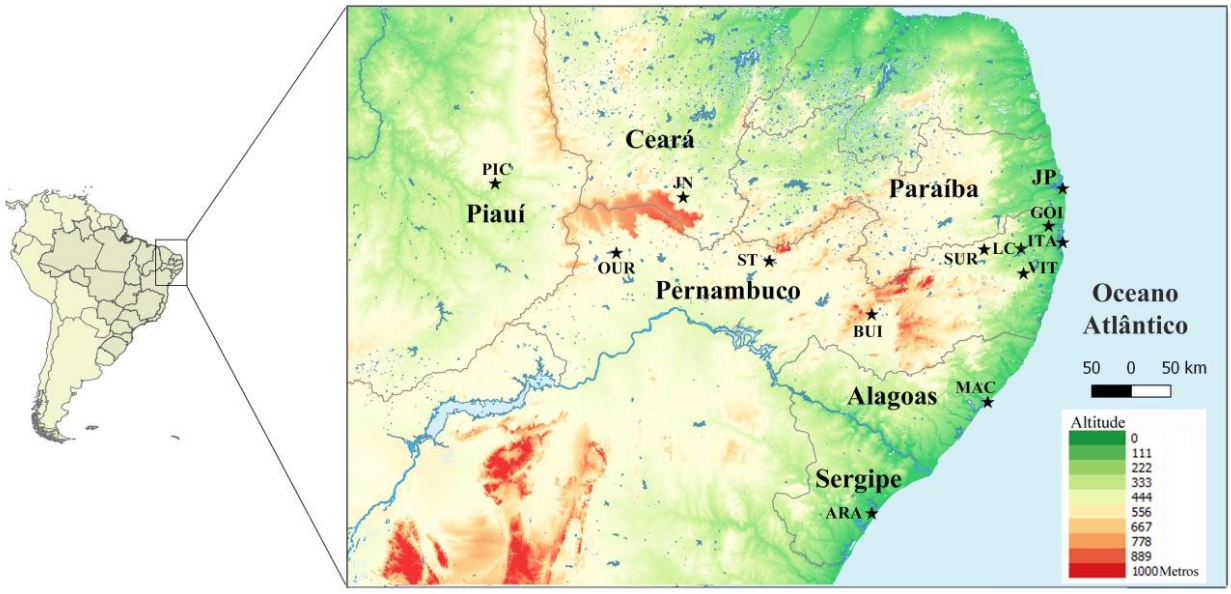

Figura 1: Populações e coordenadas geográficas onde foram amostrados o gafanhoto *R. brasiliensis*, no Nordeste brasileiro. Itamaracá (ITA-PE) 07°45'0"S; 34°05'10"W; Goiana (GOI-PE) 07º33'38"S; 35º00'9"W; Vitória de Sto A. (VIT-PE) 08º07'35"S; 35º18'27"W; Lagoa do Carro (LC-PE) 07º50'56"S; 5º19'14"W; Buíque (BUI-PE) 08º37'23"S; 37º09'21"W; Surubim (SUR-PE) 07º59'31"S; º17'54"W; Serra Talhada (ST-PE) 07º49'59"S; 35º45'17"W; Ouricuri (OUR-PE) 07º52'57"S; 40º04'54"W; Aracaju (ARA-SE) 10º59'07"S; 37º04'24"W; Maceió (MAC-AL) 09º39'59"S; 35º44'06"W; João Pessoa (JP-PB) 07º06'54"S; 4º51'47"W; Juazeiro do Norte (JN-CE) 07º12'47"S; 39º18'55"W; Picos (PIC-PI) 07º04'37"S; 41º28'01"W.

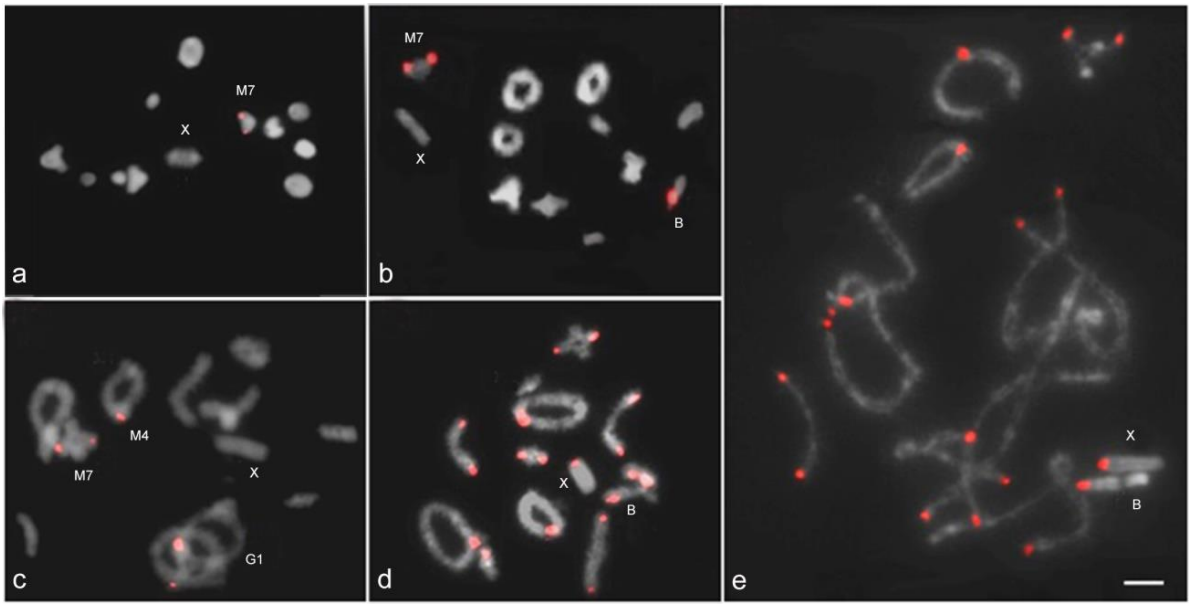

Figura 2. Hibridização *in situ* fluorescente usando sondas de histona H3 (vermelho) em *R. brasiliensis* mostrando diferentes padrões de distribuição (a, b) metáfases I, (c, d) diplótenos e (e) paquíteno. Barra = 5 µm

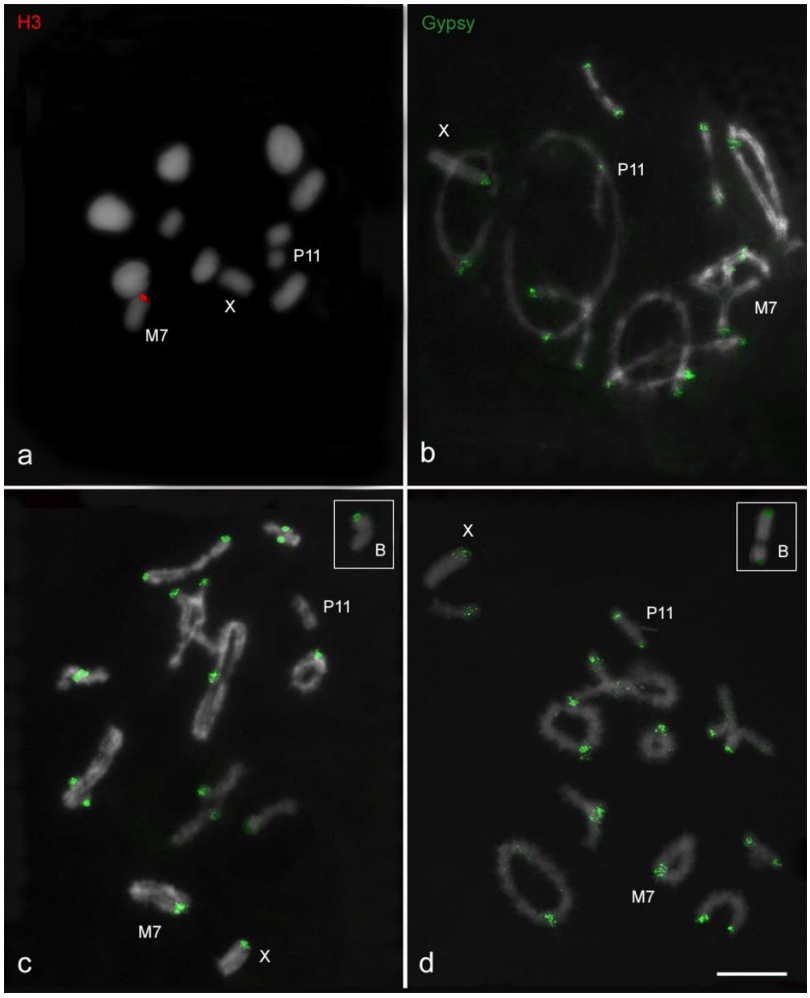

Figura 3. Hibridização *in situ* fluorescente com sondas de *Rbra_Gypsy1* (verde) em *R. brasiliensis* em indivíduos com diferentes padrões de dispersão de histona H3. (a e b) metáfase I e paquíteno, respectivamente, com marcação de H3 e *Rbra_Gypsy1*. Observe que o padrão de distribuição desses sítios em um mesmo indivíduo foi diferente; (c e d) diplótenos com marcação de *Rbra_Gypsy1* em quase ou em todos os cromossomos. Padrão de distribuição similar ao observado para H3. Os insertos destacam o cromossomo B. Barra = 5 µm

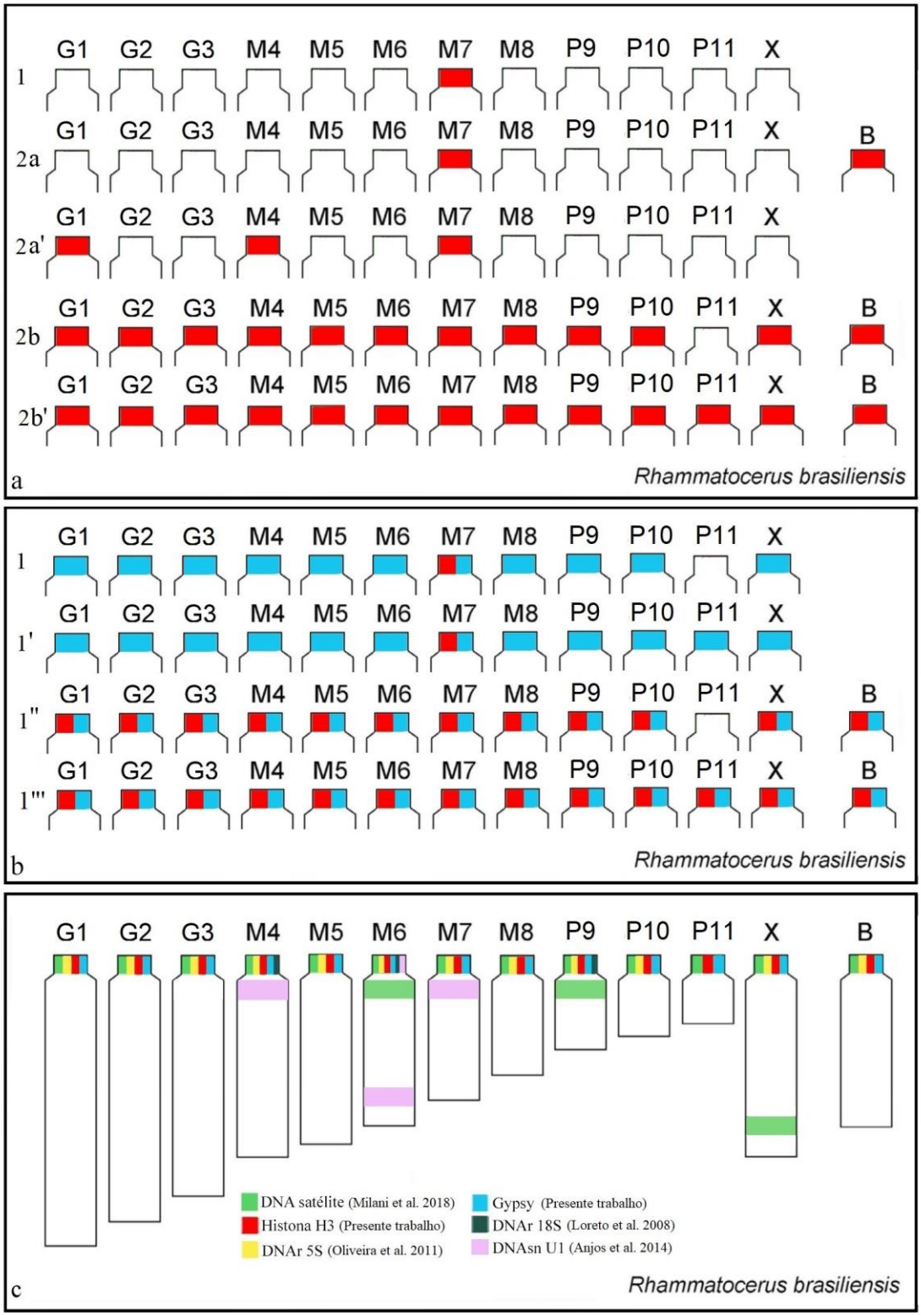

Figura 4. Ideogramas de *R. brasiliensis* mostrando os padrões de distribuição de sequências de Histona H3 e do retrotransposon *Rbra_Gypsy1* e suas variações no genoma da espécie (a, b) e de todas as sequências repetitivas mapeadas até o momento (c). (a) Em destaque os padrões de distribuição dos sítios de Histona H3, presentes apenas no M7 (1); no M7 e no cromossomo B (2a), nos bivalentes autossômicos L1, M4 e M7 (2a'); em todos os cromossomos do complemento, exceto o S11, inclusive no B (2b) e em todos os cromossomos do complemento e no B (2b'); (b) Em destaque o padrão de localização das sequências de *Rbra_Gypsy1* obtido em indivíduos também mapeados pela histona H3. Os sítios de *Rbra_Gypsy1* mostram padrão de ampla dispersão em todos os cromossomos, exceto o S11, e também com marcação no B quando presente (1 e 1''); marcação em todos os cromossomos, inclusive o B quando presente (1' e 1'''). Note que o *Rbra_Gypsy1* também está disperso em indivíduos com marcação de H3 restrita ao M7 (1 e 1'). (c) Localização de diferentes sítios de DNA repetitivo mapeados na espécie até o momento.

Tabela 1. Padrões de distribuição dos sítios de histona H3 e retrotransposon *Rbra_Gypsy1* mapeados, respectivamente, em 88 e 10 indivíduos da espécie *Rhammatocerus brasiliensis*, amostrados em Estados do Nordeste brasileiro.

	Cromossomos portadores de sítios de Histona H3 / *Rbra_Gypsy1*							
Populações	**(M7)**	**(M7 + B)**	**(3 Ba)**	**Tc (- S11)**	**Tc (- S11) +B**	**Tc**	**Tc + B**	**Total**
ITA-PE	7/-					6/-	1/-	14/-
GOI-PE	4/-			2/-		-/1	-/-	6/1
SUR-PE	6/-							6/-
VIT-PE	2/-		1/-	1/-	1/1	-/-	1/-	6/1
ST-PE	6/-					-/1	1/-	7/1
LC-PE	2/-	1/-				3/-	1/1	7/1
OUR-PE	1/-				1/-	4/-		6/-
BUI-PE	1/-					4/-	1/-	6/-
JP-PB						6/-		6/-
JN-CE	2/-					3/-	1/-	6/-
PIC-PI	2/-			-/1		3/-	1/-	6/1
MAC-AL				1/-	1/-	4/3		6/3
ARA-SE				1/1		5/1		6/2
Total	33/-	1/-	1/-	5/2	3/1	38/6	7/1	88/10

G: Grande, M: Médio, P: Pequeno, Ba: Bivalente autossômico, Tc: Todos os cromossomos, B: Cromossomo B; Números intercalados por barra indicam os indivíduos que possuem sítios de Histona H3 (esquerda da barra) e de *Gypsy* (direita da barra); - Sem resultado ou não foram mapeados para *Rbra_Gypsy1*.

5 DISCUSSÃO GERAL

Análise da dispersão do cromossomo B de *Rhammatocerus brasiliensis* em populações de estados do Nordeste brasileiro

O polimorfismo para cromossomo B presente em *R. brasiliensis* foi observado em 19 das 21 populações analisadas e distribuídas em sete estados do Nordeste brasileiro. Este dado demonstrou a ampla dispersão do cromossomo B ao longo da distribuição da espécie, além de ampliar o registro da ocorrência desse polimorfismo, que havia anteriormente sido registrado em oito localidades de Pernambuco e três na Bahia (LORETO *et al.,* 2008b). Segundo PALESTIS *et al.,* (2010), polimorfismo para cromossomo B é comum em ortópteros, correspondendo a 17,1% em gafanhotos acridídeos. Entretanto, o registro da dispersão do cromossomo B em espécies neotropicais é escasso (LORETO *et al.,* 2008b; BERNARDINO *et al.,* 2017).

A não detecção de cromossomos B em João Pessoa - PB e Aracaju - SE pode estar relacionada a baixa frequência do B nessas populações, sugerindo que o B se encontra no estágio de quase neutralidade, resultante da tendência do genoma hospedeiro de eliminar esse cromossomo. Adicionalmente, a maioria das populações analisadas não apresentaram diferença significativa quanto a ocorrência e frequência de cromossomos Bs, incluindo as populações de João Pessoa-PB e Aracaju – SE. Isto pode ser resultante da baixa frequência de B em algumas populações analisadas e ao relativo baixo número amostral das populações de João Pessoa – PB (35 indivíduos) e de Aracaju - SE (dez indivíduos), ambas com ausência de B. Os números amostrais dessas populações não foram suficientes para detectar a ocorrência do cromossomo B, de acordo com o cálculo do intervalo

de confiança que apontou frequência máxima para o polimorfismo de 8,2% em João Pessoa - PB e 26% em Aracaju- SE.

Devido à ampla distribuição de indivíduos de *R. brasiliensis* portadores de cromossomo B, fluxo gênico positivo (Nm=2,3869) e baixa diferenciação genética F_{ST} (0.15) e G_{ST} (0.17) entre as populações, sugere-se que este polimorfismo está amplamente disperso entre as populações do Nordeste brasileiro, presente em 19 das 21 populações analisadas oriundas de sete estados do Nordeste. Devido ao comportamento migratório de *R. brasiliensis* (CARBONELL, 1988) este é um padrão esperado, considerando que o provável meio para dispersão do cromossomo B em diferentes faixas geográficas, seria através do fluxo gênico entre hospedeiros e indivíduos de populações que não possuem o B (CAMACHO *et al.,* 2015). O teste de Mantel sugeriu que não há correlação entre distância genética e geográfica, mesmo entre populações que distam mais de 700Km (Itamaracá - PE e Juazeiro do Norte - CE), podendo estar contribuindo para a dispersão dos cromossomos Bs, uma vez que sua transmissão é estritamente vertical (MUNOZ *et al.,* 1998).

Os dados de variância molecular (AMOVA), maior dentro das populações (84,68%), quando atrelados aos dados do Structure corroboram com os baixos índices de diferenciação genética, onde a presença de uma mistura de dois perfis genéticos é relacionada ao hábito migratório, ampla mobilidade e capacidade de voo de *R. brasiliensis* (CARBONELL, 1988; 1995).

Baseado nas evidências descritas na literatura de que cromossomos B são transmitidos apenas de forma vertical, é sugerido que para a espécie *R. brasiliensis* a ampla distribuição do B é de fato decorrente do fluxo gênico existente entre as populações analisadas. O hábito migratório atrelado a alta capacidade de voo, além da ampla oferta de recurso alimentar nas localidades amostradas são fatores que

certamente contribuem para conectividade genética de suas populações e consecutivamente para a ampla dispersão do cromossomo B.

Adicionalmente, foram observados dois Bs nas populações de Bezerros - PE, Ouricuri - PE e Itamaracá - PE e três Bs em Itamaracá – PE. O acúmulo de Bs provavelmente é resultante da não disjunção das cromátides irmãs desse elemento durante a meiose (BANAEI-MOGHADDAM *et al.,* 2012). Além disso, ocorrem dois tipos de B, o mais comum de tamanho menor que o X e com ocorrência em todas as populações que possuem esse polimorfismo, e um segundo denominado B variante, que é de tamanho semelhante ao X e possui um *gap*.

O cromossomo B de *R. brasiliensis* possui HC restrita a região pericentromérica observada neste estudo e por Loreto *et al.,* (2008b), indicando que este cromossomo possui pouca HC, diferente de outras espécies de gafanhotos que possuem cromossomos Bs com amplificação da HC (LORETO *et al.,* 2008b; BERNARDINO *et al.,* 2017; MILANI *et al.,* 2018). Adicionalmente, o B variante presente na população de Lagoa do Carro - PE também apresentou uma região terminal C positiva. Esta marcação, além do *gap* e diferença no tamanho do cromossomo, foi mais uma evidência da diferenciação do B de *R. brasiliensis*, indicando a ocorrência de um B variante na espécie.

O cromossomo B variante de *R. brasiliensis* pode ter se originado a partir da amplificação de sequências repetitivas. Contudo, dentre elas excluímos os DNAr 45S, 5S, histona H3 (LORETO *et al.,* 2008b; OLIVEIRA *et al.,* 2011) e sequências de DNAsat (RbrSat01-171, RbrSat03-36, RbrSat04-168 e RbrSat08-176) identificadas no B da espécie (MILANI *et al.,* 2018). Essas sequências encontram-se localizadas nas regiões pericentroméricas de ambos os tipos de B. Os elementos de transposição são bons candidatos ao enriquecimento do B, visto que já foi

observado o acúmulo desse tipo de DNA repetitivo em B variante no peixe *Astatotilapia latifasciata* (COAN e MARTINS, 2018), no gafanhoto *Locusta migratoria* (RUIZ-RUANO *et al.,* 2018) e no centeio *Secale cereale* (KLEMME *et al.,* 2013).

O B variante observado em *R. brasiliensis* provavelmente se encontra no estágio de regeneração do ciclo no qual acumulou modificações para escapar da neutralização pelo genoma do hospedeiro (HENRIQUES-GIL E ARANA, 1990; CAMACHO *et al.,* 1997). A ocorrência do B variante reforça a ideia da possível origem não recente do B observado neste estudo em 19 populações e descrito por LORETO *et al.* (2008b), considerando que são necessárias sucessivas gerações para o B ser quase neutralizado, acumular modificações e se dispersar nas populações. Além disso, dada a sua frequência (0 a 18,8%, média de 8,5%), provavelmente o B encontra-se em estágio de quase neutralidade do ciclo, sendo esse o mais demorado, onde são necessárias sucessivas gerações para a dispersão do cromossomo B, que tem transmissão estritamente vertical.

Estudo do cromossomo B de *Rhammatocerus brasiliensis* através do mapeamento de sequências repetitivas, com ênfase na origem do B e dispersão da histona H3 no genoma da espécie

Os sítios de histona H3 foram mapeados em indivíduos de *R. brasiliensis* oriundos de 13 populações do Nordeste brasileiro e apresentaram dispersão no genoma de indivíduos de 11 populações. Dessas apenas em seis populações (Vitoria de Santo Antão - PE, Lagoa do carro - PE, Ouricuri - PE, Serra Talhada - PE, Picos - PI e Maceió - AL) foram analisadas células com presença do B.

Além de apresentar sequências de histona H3, o B de *R. brasiliensis* também possui quatro sequências de DNA satélite (RbrSat01-171, RbrSat03-75, RbrSat04-

168 e RbrSat08-176), sendo uma delas (RbrSat04-168) exclusiva do B e do par S11 (MILANI *et al.,* 2018), indicando que esse par está relacionado a origem do B e que sua origem é intraespecífica. A ancestralidade do cromossomo B é conhecida apenas em poucas espécies (RUIZ-RUANO *et al.,* 2017). Em gafanhotos, DNAs repetitivos têm sido usados para rastrear a origem e composição do cromossomo B em relativamente poucas espécies. Em *E. monticola*, por exemplo, a ascendência do cromossomo B é atribuída ao par autossômico P8 baseado na análise de satDNAs (RUIZ-RUANO *et al.,* 2017). Em *A. flavolineata*, a origem do cromossomo B do par 1 é atribuída à presença única de genes de snDNA U2 nesses dois cromossomos (BUENO *et al.,* 2013). A ancestralidade do cromossomo B em *L. migratória* está relacionada aos pares 8 e 9, devido à presença de satDNAs e genes de histonas nesses cromossomos (TERUEL *et al.,* 2010; RUIZ-RUANO *et al.,* 2018).

Embora nossas descobertas apoiem fortemente a ancestralidade do cromossomo B do par S11 em *R. brasiliensis* (pelo menos na população de Lagoa do Carro / PE), devemos também chamar a atenção para o par M7 que compartilha três DNAsat com o cromossomo B. Em outras populações (incluindo Lagoa do Carro / PE), o cromossomo M7 abriga o gene da histona H3, que em um indivíduo é compartilhado exclusivamente com o cromossomo B. Isto sugere o envolvimento de M7, além do par S11, na origem do B.

Similar a *L. migratória,* a ancestralidade do cromossomo B é supostamente de dois cromossomos, os pares 8 e 9 (TERUEL *et al.,* 2010; RUIZ-RUANO *et al.,* 2018). Por outro lado, devemos ter em mente que o par M7 abriga um DNAsat (RbrSat05-179) que não é observado no cromossomo B. Além disso, considerando o alto dinamismo do gene da histona H3 (em número de *clusters*) em *R. brasiliensis*, é possível que esse gene tenha sido posteriormente adquirido pelo cromossomo B.

A presença dos sítios de histona H3 restrita ao par M7 no gafanhoto *R. brasiliensis* em 33 indivíduos, em dez das 13 populações aqui analisadas, bem como nos indivíduos que apresentaram a dispersão da H3, sugere que o sítio ancestral dessa sequência se encontra nesse bivalente, tendo se dispersado para outros cromossomos posteriormente. A localização da H3 em um par cromossômico de tamanho médio corrobora com o observado em outras espécies de acridídeos estudadas (CABRERO *et al.,* 2009; PALACIOS-GIMENEZ *et al.,* 2013; SILVA-NETO *et al.,* 2015; CASTILLO *et al.,* 2017; PINE *et al.,* 2017), que apresentaram conservação da localização cromossômica de histonas, e que segundo CABRERO *et al.* (2009) essas sequências não sofreram alterações significativas desde a origem do grupo a cerca de 60 milhões de anos. A presença do sítio de histona ocorre com maior frequência em um dos pares autossômicos grandes (2 ou 3) ou médios (5 ou 8) nos acridídeos e possivelmente sofre a ação de uma forte seleção purificadora atuando para manter a localização dessa sequência conservada, no genoma de espécies de gafanhotos (CABRERO *et al.,* 2009; PALACIOS-GIMENEZ *et al.,* 2013; SILVA-NETO *et al.,* 2015; CASTILLO *et al.,* 2017; PINE *et al.,* 2017).

O número de *cluters* dos genes de histona H3 parece ser altamente conservado não só em gafanhotos da família Acrididae, como também na família Proscopiidae, a segunda família mais antiga de gafanhotos (CABRAL-DE-MELLO *et al.,* 2011c) e em outros grupos de insetos, como besouros do gênero *Dichotomius* (CABRAL-DE-MELLO *et al.,* 2011b) e em *Lagria villosa* (GOLL *et al.,* 2015), algumas espécies de mariposa (ŠÍCHOVÁ *et al.,* 2013), percevejo (BARDELLA *et al.,* 2016) e pulgão (MANDRIOLI E MANICARDI 2013), espécies de cigarrinha (ANJOS *et al.,* 2018). Contudo, a ocorrência de número de *cluster* de histona H3 presente em mais de um bivalente cromossômico têm sido relatada em espécies de

diferentes grupos de invertebrados, como grilo *Gryllus assimilis* (PALACIOS-GIMENEZ *et al.,* 2015), pulgão *Diuraphis noxia* (NOVOTNÁ *et al.,* 2011), besouro *Euchroma gigantea* (XAVIER *et al.,* 2018), escorpião *Tityus obscurus* (ALMEIDA *et al.,* 2017), espécies de molusco (GARCÍA-SOUTO *et al.,* 2015) e nos acridídeos *Dichromatos lilloanus, Abracris flavolineata* e *Ronderosia bergi* (BUENO *et al.,* 2013; PALACIOS-GIMENEZ *et al.,* 2013, 2015).

Rammathocerus brasiliensis apresentou variação no padrão de dispersão da histona H3, de moderada a ampla. Os dados observados indicam que a dispersão da sequência de H3 ocorreu a partir da região pericentromérica do bivalente M7 para os demais cromossomos, com a presença de sítios em mais um ou dois cromossomos até a dispersão completa (sítios em todos os cromossomos, inclusive o B). Os DNAs repetitivos presentes nas regiões heterocromáticas podem desempenhar um papel importante na dispersão de sequências, podendo promover variação na distribuição de sequências de histona entre indivíduos de *R. brasiliensis* de diferentes populações. Isto é plausível, visto que os genomas de gafanhotos possuem grande quantidade de sequências repetitivas, como observado em *Locusta migratoria*, que possui maior abundância para elementos repetitivos, que constituem cerca de 60% do genoma, sendo cerca de 24% transposons de DNA e 17% retrotransposons LINE (WANG *et al.,* 2014).

Como a histona H3 foi localizada na região pericentromérica dos cromossomos de *R. brasiliensis*, que é rica em outras sequências repetitivas, como: DNAr 18S e 5S (LORETO *et al.,* 2008b; OLIVEIRA *et al.,* 2011), DNAsn U1 (ANJOS *et al* 2014) DNA satélite (MILANI *et al.,* 2018) e o TE *Rbra_Gypsy1* mapeado neste estudo (Figura 4c, Tabela 1), é possível supor a participação do *Rbra_Gypsy1* na dispersão dessas sequências, em especial dos sítios de H3, devido a sua

localização semelhante na região pericentromérica em todos os cromossomos, exceto o S11, ou em todos os cromossomos, incluindo o B. O TE *Rbra_Gypsy1* pode estar resultando na dispersão das sequências de H3 através da promoção de regiões com alta homologia devido a sua inserção em novos locais no genoma *R. brasiliensis*, que consecutivamente deve ter levado a ocorrência de recombinações ectópicas entre os cromossomos (CRAIG *et al.,* 2002; MACKAY 2007; SANTANA *et al.,* 2014; MAUMUS *et al.,* 2015). Este elemento *Rbra_Gypsy1* pode ainda estar relacionado a funções estruturais nos centrômeros dos cromossomos da espécie *R. brasiliensis*, o que justificaria sua localização preferencial pela região pericentromérica (PLOHL *et al.,* 2014).

Tabela 1. Localização das sequências repetitivas mapeadas *em Rhammatocerus brasiliensis*

Sequência Repetitiva	**Localização Cromossômica**													
	1	2	3	4	5	6	7	8	9	10	11	X	B	Referências
DNAr 18S				p		p			p					Loreto *et al.,* (2008b); Oliveira *et al.,* (2011)
DNAr 5S		p	p,i		p						p		p	Loreto *et al.,* 2008b
DNAr 5S	p	p	p	p	p	p	p	p	p	p		p	p	Oliveira *et al.,* 2011
Histona H3	p	p	p	p	p	p	p	p	p	p		p	p	Oliveira *et al.,* 2011
Histona H3							p							Neste estudo
Histona H3							p						p	
Histona H3	p			p			p							
Histona H3	p	p	p	p	p	p	p	p	p	p		p		
Histona H3	p	p	p	p	p	p	p	p	p	p		p	p	
Histona H3	p	p	p	p	p	p	p	p	p	p	p	p		
Histona H3	p	p	p	p	p	p	p	p	p	p	p	p	p	
Gypsy	p	p	p	p	p	p	p	p	p	p		p		
Gypsy	p	p	p	p	p	p	p	p	p	p		p	p	
Gypsy	p	p	p	p	p	p	p	p	p	p	p	p		
Gypsy	p	p	p	p	p	p	p	p	p	p	p	p	p	
DNAsat RbrSat01-171	p	p			p		p	p	p		p		p	Milani *et al.,* 2018
DNAsat RbrSat02-410							nc							
DNAsat RbrSat03-36	p	p	p	p	p	p	p	p	p	p	p	p	p	
DNAsat RbrSat04-168											p		P	
DNAsat RbrSat05-179							p		p					
DNAsat RbrSat06-165							nc							
DNAsat RbrSat07-240							nc							
DNAsat RbrSat08-176			p	p		p,i	p			p	p	i	p	
DNAsat RbrSat09-238									i					
DNAsat RbrSat10-268							nc							
DNAsat RbrSat11-233							nc							
DNAsat RbrSat12-180							nc							

p: pericentromêrica, i: intesticial, nc: não clusterizado.

6 CONCLUSÕES

1. O compartilhamento de quatro sequências de DNAsat entre o par S11 e o cromossomo B em *R. brasiliensis*, sendo uma delas exclusiva (RbrSat04-168) a esse par e o B, sugere que o B se originou a partir do S11, sendo sua origem portanto autossômica e intraespecífica.
2. O cromossomo B de tamanho pequeno, no gafanhoto *R. brasiliensis,* apresenta ampla ocorrência e dispersão em populações da espécie no Nordeste brasileiro, favorecidas pela conectividade genética entre as populações. Devido aos valores e média de sua frequência (0 a 18,8%, média de 8,5%) nas populações, provavelmente esse cromossomo B encontra-se em estágio de quase neutralidade do ciclo.
3. O B variante (tamanho médio, com *gap* e HC adicional terminal) presente em três populações de *R. brasiliensis,* provavelmente se encontra no estágio de regeneração do ciclo, no qual acumulou modificações para escapar da neutralização pelo genoma do hospedeiro.
4. O par cromossômico autossômico M7 é portador do sítio primitivo de histona H3 na espécie *R. brasiliensis*. A observação da dispersão dos sítios de histona H3 nos cromossomos de *R. brasiliensis* em quase todas as populações do Nordeste brasileiro analisadas, possivelmente é resultante de recombinação ectópica favorecida pela presença do retrotransposon *Gypsy* nas regiões cromossômicas pericentroméricas na espécie. Contudo, pode ser também devido a mecanismos de retrotransposição que podem ter amplificado, dispersado e integrado cópias de sequências H3.

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APÊNDICE A

genes

MDPI

Article

Satellite DNAs Unveil Clues about the Ancestry and Composition of B Chromosomes in Three Grasshopper Species

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Abstract: Supernumerary (B) chromosomes are dispensable genomic elements occurring frequently among grasshoppers. Most B chromosomes are enriched with repetitive DNAs, including satellite DNAs (satDNAs) that could be implicated in their evolution. Although studied in some species, the specific ancestry of B chromosomes is difficult to ascertain and it was determined in only a few examples. Here we used bioinformatics and cytogenetics to characterize the composition and putative ancestry of B chromosomes in three grasshopper species, *Rhammatocerus brasiliensis*, *Schistocerca rubiginosa*, and *Xyleus discoideus angulatus*. Using the RepeatExplorer pipeline we searched for the most abundant satDNAs in Illumina sequenced reads, and then we generated probes used in fluorescent in situ hybridization (FISH) to determine chromosomal position. We used this information to infer ancestry and the events that likely occurred at the origin of B chromosomes. We found twelve, nine, and eighteen satDNA families in the genomes of *R. brasiliensis*, *S. rubiginosa*, and *X. d. angulatus*, respectively. Some satDNAs revealed clustered organization on A and B chromosomes varying in number of sites and position along chromosomes. We did not find specific satDNA occurring in the B chromosome. The satDNAs shared among A and B chromosomes support the idea of putative intraspecific ancestry from small autosomes in the three species, i.e., pair S11 in *R. brasiliensis*, pair S9 in *S. rubiginosa*, and pair S10 in *X. d. angulatus*. The possibility of involvement of other chromosomal pairs in B chromosome origin is also hypothesized. Finally, we discussed particular aspects in composition, origin, and evolution of the B chromosome for each species.

Keywords: fluorescent in situ hybridization; Orthoptera; satellite DNA; supernumerary chromosome; RepeatExplorer

Dados do presente trabalho referente à espécie *Rhammatocerus brasiliensis*

1. Introduction

Eukaryotic genomes exhibit repetitive DNA sequences including noncoding tandemly repeated satellite DNA (satDNA). These sequences exhibit extensive variability in copy number and nucleotide sequence, even among phylogenetically related species. Arrays of satDNAs are usually located in the centromeric and telomeric heterochromatin of the chromosomes, although they have also been reported in the euchromatic region. Furthermore, satDNAs are frequently enriched on sex chromosomes and supernumerary (B) chromosomes, as they are greatly enriched in heterochromatin [1–3].

Supernumerary B chromosomes occur in approximately 15% of eukaryotes as dispensable elements (i.e., not required for normal organismal development), frequently heterochromatic and enriched repetitive DNAs, including the satDNAs, which can have implications for B chromosome evolution. Generally, B chromosomes do not recombine with A chromosomes (normal complement), and B chromosome sequences evolve at a higher evolutionary rate than A elements [4,5]. Since the first discovery of the B chromosome [6], the specific ancestry of the studied B chromosomes in eukaryotes has remained largely unknown. For decades, repetitive DNAs have been used to try to ascertain the ancestry and to describe the B chromosome composition in some species. In that way, satDNAs have helped the understanding of the evolutionary history of B chromosomes with intraspecific (from host genome) or interspecific (resultant of species hybridization) origin, for example, in grasshoppers [7,8], wasps [9], fish [10], and plants [11], among others.

Among the grasshoppers, approximately 12% of the species harbor B chromosomes. Some families seem to be hotspots for B chromosome presence, such as Acrididae, with 17.1% of the species harboring B chromosomes, unlike Romaleidae in which only 4% of the species is harboring B chromosomes [12]. As generally observed in eukaryotes, some repetitive DNAs populate the B chromosomes of grasshoppers, like the multigene families for rDNAs [13,14], histone genes [14,15], and U snDNA [16], transposable elements [8,17,18], microsatellites [19], and satDNAs [7,8,13,20]. These sequences shed light on B chromosome composition, variability, and evolutionary dynamics. Concerning satDNAs, their presence in the B chromosomes of grasshoppers is known only in a few species, including *Locusta migratoria* [8], *Abracris flavolineata* [20], *Eyprepocnemis plorans* [13], and *Eumigus monticola* [7].

The search for satDNAs in genomic data was facilitated more recently by analyzing reads from next generation sequencing (NGS) using bioinformatics approaches, like RepeatExplorer software [21]. RepeatExplorer has been a useful tool for detecting satDNAs for probe generation and chromosome mapping in species with B chromosomes, helping to unveil the composition and abundance of satDNAs in those chromosomes as well as their relationships with A elements as well [7,8,11,22].

By combining genomics and cytogenetics, we aimed to elucidate the genome content of satDNAs and used this information to track the possible ancestry of B chromosomes in three grasshopper species, *Rhammatocerus brasiliensis* (Acrididae: Gomphocerinae), *Schistocerca rubiginosa* (Acrididae: Cyrtacanthacridinae), and *Xyleus discoideus angulatus* (Romaleidae: Romaleinae) belonging to two families. The family Acrididae, which is currently most diverse lineage within the orthopteran suborder Caelifera, diverged from its sister lineage, which includes the family Romaleidae, in the late Cretaceous (~78 mya, million years ago) based on a fossil-calibrated divergence time estimate. Two acridid subfamilies included in this study, Gomphocerinae and Cyrtacanthacridinae, each belong to different clades within the family, and they are estimated to have diverged in the late Eocene [23]. For this purpose, we first made a prediction of the most abundant satDNAs in genomes by using the RepeatExplorer tool. Then we recovered the fragments by PCR and designed probes of each satDNA of the three species to use in fluorescent in situ hybridization (FISH) experiments. This allows for the investigation of spatial patterns of satDNAs that have preferentially accumulated in B chromosomes compared to autosomes. We found distinct patterns of satDNA distribution on B chromosomes that are shared with some A chromosomes or with exclusive A chromosomes. Based on these data, it is possible to hypothesize the ancestry of the B chromosome from small autosomes and to discuss aspects of the evolution of B chromosomes in the three species.

2. Material and Methods

2.1. Animal Sampling, Chromosome Preparations, and Genomic DNA Sequencing

Adult animals of *R. brasiliensis* were collected at 07°45′00″ S; 34°05′10″ W Ilha de Itamaracá/PE (Brazil) and 07°50′56″ S 35°19′14″ W Lagoa do Carro/PE (Brazil); *X. d. angulatus* at 07°12′47″ S 39°18′55″ W Juazeiro do Norte/CE (Brazil); *S. rubiginosa* at 29°25.908′ N 82°24.060′ W Levy County/Florida (USA). Testes were fixed with Carnoy's modified solution (3:1, 100% Ethanol:Glacial Acetic Acid) and stored at −20 °C until use for slides preparation. Femurs were immersed in 100% ethanol and stored at −20 °C for genomic DNA (gDNA) extraction.

The genomic DNA sequencing method for the *S. rubiginosa* (male) specimen was previously described [24]. For the *R. brasiliensis* (female) and *X. d. angulatus* (male) specimens DNA extraction was performed using the phenol/chloroform-based procedure described previously [25]. Sequencing was conducted by the Illumina company (Inc., San Diego, CA, USA) with a HiSeq 4000 to obtain paired-ends libraries (2 × 101 bp) using the service of Macrogen Inc. (Seoul, Republic of Korea).

We applied conventional staining with 5% Giemsa for chromosome observation and identification of individuals harboring B chromosomes. The C-banding for heterochromatin identification was performed according to a previously described method [26].

2.2. SatDNAs Searching by Graph-Based Clustering Method

Prior to RepeatExplorer graph-based clustering analysis, we preprocessed and checked the quality of the paired-ends reads of each species using FastQC [27]. Preprocessing of the reads was performed following default parameters using the public online platform: https://repeatexplorer-elixir.cerit-sc.cz/galaxy/. Reads were processed with a "quality trimming tool", "FASTQ interlacer" on paired end reads, "FASTQ to FASTA" converter, and "RepeatExplorer clustering" all with default recommended options [21]. First we searched and selected, by visual observation, the clusters that showed high graph density that indicated proximity with satDNAs families [28]. Contigs from the selected clusters were deeply explored and manually searched for sequences with tandem pattern confirmed by the dot plot graphics implemented using Geneious v4.8.5 software [29]. The consensus monomer of each satDNA family of each species was used as the query in two databanks, BLAST (http://www.ncbi.nlm.gov/Blast/) and Repbase (http://www.girinst.org/repbase/), to check similarity with another sequence deposited and described. Abundance of each satDNA family was calculated with the number of reads of each cluster divided by the total number of reads used in the "RepeatExplorer clustering" protocol [21]. Nucleotide divergence was calculated using the RepeatMasker package with specific parameters provided in the scripts program protocol to calculate Kimura divergence values [30]. Superfamilies (SF) were considered by comparing consensus monomer of each satDNA against all of them from each species independently using the Geneious v4.8.5 software [29] assembly tool, alternating overlap identity following the same considerations as a previous work [31]. We classified each identified satDNA family according to a previous method [31], considering the species name abbreviation and decreasing abundance, followed by the consensus monomer size; they were numbered in decreasing order of abundance. The sequences were deposited in GenBank under the accession numbers MH900339–MH900377.

2.3. Amplification of SatDNAs through PCR, Probes and Fluorescence In Situ Hybridization

We used the consensus sequences of each satDNA family of each species to design divergent primers manually or using the Primer3 tool [32] implemented in Geneious v4.8.5 software [29] (Supplementary Table S1). Polymerase chain reactions (PCRs) were performed using 10× PCR Rxn Buffer, 0.2 mM $MgCl_2$, 0.16 mM dNTPs, 2 mM of each primer, 1 U of *Taq* Platinum DNA Polymerase (Invitrogen, San Diego, CA, USA), and 50–100 ng/μL of template DNA. The PCR conditions included an initial denaturation at 94 °C for 5 min and 30 cycles at 94 °C (30 s), 55 °C (30 s), and 72 °C (80 s), plus a final extension at 72 °C for 5 min. The PCR products were visualized on a 1% electrophoresis agarose

gel. The monomeric bands were isolated and purified using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research Corp., The Epigenetics Company, CA, USA) according to the manufacturer's recommendations and then used as template for reamplification using the same PCR conditions. The monomers were sequenced by the Sanger method using the service of Macrogen Inc. to confirm the amplification of desired sequence.

FISH was performed in meiotic chromosomes using one or two probes according to a method described previously [33] with some adjustments as outlined previously [34]. The probes labeled with digoxigenin-11-dUTP were detected using anti-digoxigenin rhodamine (Roche, Mannheim, Germany), and probes labeled with biotin-14-dATP were detected using Streptavidin Alexa Fluor 488-conjugated (Invitrogen, San Diego, CA, USA). The preparations were counterstained using 4′,6-diamidine-2′-phenylindole (DAPI) and mounted in VECTASHIELD (Vector, Burlingame, CA, USA). FISH results were observed using an Olympus microscope BX61 (Tokyo, Japan) equipped with a fluorescent lamp and the proper filters. Images were obtained using a DP71 cooled digital camera in grayscale and then pseudo-colored in blue for chromosomes and red or green for hybridization signals, merged and optimized for brightness and contrast using Adobe Photoshop CS6. To describe the patterns of satDNA chromosomal distribution distinct cells were analyzed, including diplotene, metaphase I, metaphase II, and mitotic metaphase.

3. Results

3.1. Karyotypes, B Chromosomes, and Heterochromatin Distribution

Occurrence of a karyotype consisting of 2n = 23,X0, and presence of B chromosomes observed here for *R. brasiliensis* and *X. d. angulatus* were previously reported by different authors [14,35], including in the same population, i.e., Juazeiro do Norte/CE for *X. d. angulatus* [36]. We report for the first time the presence of B chromosomes in *R. brasiliensis* from Lagoa do Carro/PE. The karyotype of *S. rubiginosa*, described here for the first time, is also 2n = 23,X0 as observed for other species from the same genus, like *S. gregaria* [37], *S. pallens*, and *S. flavofasciata* [38]. Among the five individuals of *S. rubiginosa*, two presented B chromosomes. We classified autosomal chromosomes of the three species in three distinct groups considering size: three long chromosomes (L1–L3), five medium (M4–M8), and three small (S9–S11).

The B chromosomes of the three species are acrocentric with variable pattern of heterochromatin distribution (Figure 1). For *R. brasiliensis* pericentromeric and distal blocks were observed (Figure 1a) and for *S. rubiginona* pericentromeric and interstitial blocks, close to the centromere, were noticed (Figure 1b). In *X. d. angulatus* the B chromosome was completely heterochromatic with deeper staining in the pericentromeric region (Figure 1c). Heterochromatin blocks restricted to pericentromeric areas were noticed for A chromosomes (Figure 1).

3.2. In Silico SatDNA Analysis

By using RepeatExplorer we predicted the most abundant satDNAs as follows, twelve, nine, and eighteen satDNA families in *R. brasiliensis*, *S. rubiginosa*, and *X. d. angulatus*, respectively. Monomer lengths varied from 36 to 410 nt in *R. brasiliensis*, from 107 to 441 nt in *S. rubiginosa*, and from 8 to 289 in *X. d. angulatus*. The predominance of families with monomer length higher than 100 nt was noticeable. Only for *X. d. angulatus*, satDNA families with monomer length smaller than 50 nt was observed (Table 1). Sequence similarity analysis revealed the presence of two similar satDNAs families in the genome of *R. brasiliensis*, RbrSat01-171 and RbrSat04-168 (superfamily SF1), In *X. d. angulatus* two superfamilies were noticed each composed by two satDNA families, SF1 (XanSat05-267 and XanSat07-279) and SF2 (XanSat09-130 and XanSat14-128). No similarity between satDNAs was noticed between species.

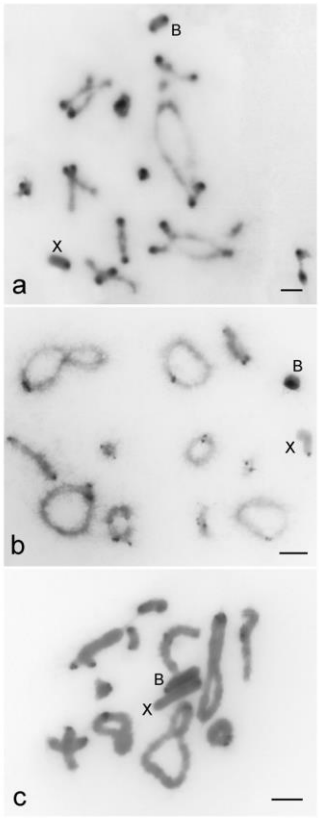

Figure 1. C-banding revealing the heterochromatin location in diplotene chromosomes of *Rhammatocerus brasiliensis* (**a**), *Schistocerca rubiginosa* (**b**), and *Xyleus discoideus angulatus* (**c**). Note the pericentromeric location of C-positive blocks on A chromosomes and the distinct patterns for the B chromosome of the three species, i.e., pericentromeric and distal in *R. brasiliensis*; pericentromeric and interstitial (close to the centromere) in *S. rubiginosa*; along the entire chromosome with darker band in pericentromeric region in *X. d. angulatus*. The X and B chromosomes are indicated. Bar = 5 µm.

A+T content was variable from 44.5% to 63.9% (mean 57.63%) in *R. brasiliensis*, from 48.9% to 61.7% (mean 55.12%) in *S. rubiginosa*, and from 28.6% to 76.2% in *X. d. angulatus* (mean 59.15%). Predominance of A+T-rich satDNA families was observed, ten in *R. brasiliensis*, eight in *S. rubiginosa*, and fifteen in *X. d. andgulatus*. Concerning total abundance, the satDNAs represented 1.499% of the genome of *R. brasiliensis*, 2.172% of *S. rubiginosa*, and 2.322% of *X. d. angulatus* genomes. In all species, even the most abundant satDNA family represented less than 1% of the genome, i.e., 0.766% in *R. brasiliensis*, 0.73% in *S. rubiginosa*, and 0.627% in *X. d. angulatus*. The lowest abundance satDNA in *R. brasiliensis* corresponded to 0.01% of the genome, in *S. rubiginosa* to 0.026%, and in *X. d. angulatus* to 0.013% (Table 1).

3.3. Chromosomal Location of SatDNAs

All satDNA families recognized by RepeatExplorer analysis were accurately amplified by PCR and sequenced; FISH mapping showed signals for most of them (Table 2; Figures 2–4). Six satDNA families revealed signals for *R. brasiliensis* (Figure 2) and *S. rubiginosa* (Figure 3), and for *X. d. angulatus* eleven satDNA families allowed identification of specific marks by FISH (Figure 4), representing clustered satDNAs. For the remaining satDNA families, six for *R. brasiliensis*, three for *S. rubiginosa*, and seven for *X. d. angulatus*, were nonclustered with no FISH signals (Table 2).

Table 1. Characteristics of satellite DNA (satDNA) families isolated from the genomes of three grasshopper species, including their monomer sizes, base pair richness, and genome abundances.

Species	SatDNA Superfamily	SatDNA Family	Monomer Size (nt)	A+T (%)	Abundance (%)	Divergence (%)
R. brasiliensis	SF1	RbrSat01-171	171	59.3	0.766	4.98
	-	RbrSat02-410	410	47.9	0.224	8.94
	-	RbrSat03-36	36	44.5	0.126	8.45
	SF1	RbrSat04-168	168	59.4	0.105	18.14
	-	RbrSat05-179	179	59.1	0.061	4.96
	-	RbrSat06-165	165	59.2	0.056	1.23
	-	RbrSat07-240	240	60.4	0.047	11.56
	-	RbrSat08-176	176	58.0	0.042	6.27
	-	RbrSat09-238	238	63.9	0.025	8.75
	-	RbrSat10-268	268	62.7	0.021	7.79
	-	RbrSat11-233	233	58.0	0.016	7.85
	-	RbrSat12-180	180	57.8	0.010	2.06
Total					1.499	
S. rubiginosa	-	SruSat01-194	194	58.3	0.730	4.30
	-	SruSat02-170	170	54.4	0.476	4.64
	-	SruSat03-170	170	59.9	0.287	7.79
	-	SruSat04-301	301	48.9	0.244	4.77
	-	SruSat05-441	441	50.6	0.135	23.18
	-	SruSat06-363	363	57.1	0.126	9.61
	-	SruSat07-232	232	61.2	0.116	9.23
	-	SruSat08-172	172	58.2	0.032	16.24
	-	SruSat09-107	107	61.7	0.026	10.85
Total					2.172	
X. d. angulatus	-	XanSat01-8	8	62.5	0.627	4.62
	-	XanSat02-21	21	28.6	0.586	4.41
	-	XanSat03-10	10	60.0	0.464	9.38
	-	XanSat04-10	10	60.0	0.228	5.22
	SF1	XanSat05-267	267	56.7	0.087	5.50
	-	XanSat06-168	168	64.3	0.069	4.53
	SF1	XanSat07-279	279	60.2	0.053	11.08
	-	XanSat08-16	16	56.2	0.033	4.38
	SF2	XanSat09-130	130	63.1	0.024	10.61
	-	XanSat10-289	289	60.2	0.022	7.27
	-	XanSat11-51	51	47.1	0.019	3.97
	-	XanSat12-246	246	59.2	0.018	11.81
	-	XanSat13-281	281	56.3	0.018	4.61
	SF2	XanSat14-128	128	62.5	0.017	14.90
	-	XanSat15-228	228	59.7	0.017	5.18
	-	XanSat16-21	21	42.9	0.014	9.79
	-	XanSat17-15	15	53.4	0.013	11.56
	-	XanSat18-21	21	76.2	0.013	6.33
Total					2.322	

The distinct clustered satDNA families were variable in number and position within A chromosomes (Figures 2–4). Only one satDNA was located exclusively on pericentromeric regions of A chromosomes in each species, RbrSat03-36 in *R. brasiliensis* (Figure 2b), SruSat02-170 in *S. rubiginosa* (Figure 3b), and XanSat03-10 in *X. d. angulatus* (Figure 4c). Heterochromatin blocks, like centromeres, were enriched in most satDNAs, but we also noticed a few satDNAs placed on the euchromatin of some chromosomes of *R. brasiliensis*: RbrSat08-176 (Figure 2e) and RbrSat09-238 (Figure 2f), and *S. rubiginosa*: SruSat03-170 (Figure 3c), SruSat06-363 (Figure 3d), SruSat07-232 (Figure 3b), and SruSat08-172 (Figure 3e). We observed that satDNA was more frequently distributed within the interstitial and distal euchromatin of *X. d. angulatus* (Figure 4a,c–g,i). The bias for pericentromeric position of satDNA was noticed by comparing the number of pericentromeric blocks with interstitial

and distal ones for each species: *R. brasiliensis* twenty-eight pericentromeric and three interstitial; *S. rubiginosa* twenty-five pericentromeric, four interstitial, and five distal; *X. d. angulatus* forty-two pericentromeric, ten interstitial, and twelve distal (Table 2).

Table 2. Chromosome location of satDNAs in three grasshopper species. For each species at the bottom is indicated the number of satDNA families per chromosome and the amount of satDNAs shared with the B chromosome. p: pericentromeric, i: interstitial, d: distal, nc: nonclustered.

Species	SatDNA Family	Chromosome Location												
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**X**	**B**
Rhammatocerus brasiliensis	RbrSat01-171	p	p			p		p	p	p		p		p
	RbrSat02-410							nc						
	RbrSat03-36	p	p	p	p	p	p	p	p	p	p	p	p	p
	RbrSat04-168											p		p
	RbrSat05-179							p		p				
	RbrSat06-165							nc						
	RbrSat07-240							nc						
	RbrSat08-176			p	p		p,i	p			p	p	i	p
	RbrSat09-238									i				
	RbrSat10-268							nc						
	RbrSat11-233							nc						
	RbrSat12-180							nc						
Total		2	2	2	2	2	2	4	2	4	2	4	2	4
shared with B		2	2	2	2	2	2	3	2	2	2	4	2	
Schistocerca rubiginosa	SruSat01-194						d							i
	SruSat02-170	p	p	p	p	p	p	p	p	p	p	p	p	i
	SruSat03-170	p,d		p,d		p			p	p	p	p		i
	SruSat04-301							nc						
	SruSat05-441							nc						
	SruSat06-363									i,d				2i
	SruSat07-232									i,d				i
	SruSat08-172			p	i	p	p			p	p,i	p		p
	SruSat09-207							nc						
Total		2	1	3	2	3	3	1	2	5	3	3	1	6
shared with B		2	1	3	2	3	3	1	2	5	3	3	1	
Xylleus discoideus angulatus	XanSat01-8	i	i	d	i	d	i	d	d	d	d	d	2i	
	XanSat02-21	p	p	p	p	p	p	p	p	p	p,d	p	p	
	XanSat03-10	p	p	p	p	p	p	p	p	p	p	p	p	p,i,d
	XanSat04-10			p			p	p						
	XanSat05-267						p				p,i			2i
	XanSat06-168					p	i							
	XanSat07-279	i	d				p							
	XanSat08-16						d							
	XanSat09-130							nc						
	XanSat10-289							nc						
	XanSat11-51		d											
	XanSat12-246	p	p	p	p	p	p	p		p	p	p		
	XanSat13-281							p		d	i			i,d
	XanSat14-128							nc						
	XanSat15-228							nc						
	XanSat16-21							nc						
	XanSat17-15							nc						
	XanSat18-21							nc						
Total		5	6	5	4	5	9	6	3	5	6	4	3	3
shared with B		1	1	1	1	1	2	2	1	2	3	1	1	

SatDNA unique to a specific A chromosome was a rare condition in the three species. It was noticed in *R. brasiliensis* for RbrSat04-168 (Figure 2c) and RbrSat09-238 (Figure 2f) in pair S11 and S9, respectively; SruSat01-194 (Figure 3a) in pair 6, and SruSat06-363 (Figure 3d) and SruSat07-232 (Figure 3b) in pair S9 of *S. rubiginosa*; in *X. d. angulatus* the repeats XanSat08-16 (Figure 4f) and XanSat11-51 (Figure 4g) were exclusive from the pairs M6 and L2, respectively. The number of

satDNAs per specific A chromosome varied from 2 to 4 (mean 2.5) in *R. brasiliensis*, from 1 to 5 (mean 2.42) in *S. rubiginosa*, and from 3 to 9 (mean 5.08) in *X. d. angulatus*.

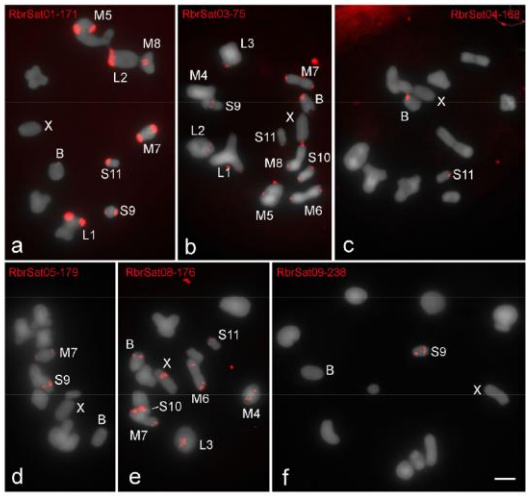

Figure 2. Fluorescent in situ hybridization (FISH) mapping on metaphase I of satDNAs identified in the genome of *R. brasiliensis*. The distinct satDNAs families are indicated. Chromosomes with signals, X and B chromosomes are identified. Note the presence on B chromosome of signals for (**a**) RbrSat01-171 (shared with some A chromosomes), (**b**) RbrSat03-36 (shared with all A chromosomes), (**c**) RbrSat04-168 (shared exclusively with pair S11), and (**e**) RbrSat08-176 (shared with some A chromosomes). For satDNAs (**d**) RbrSat05-179 and (**f**) RbrSat09-238 no signals were observed in B chromosome. Bar = 5 μm.

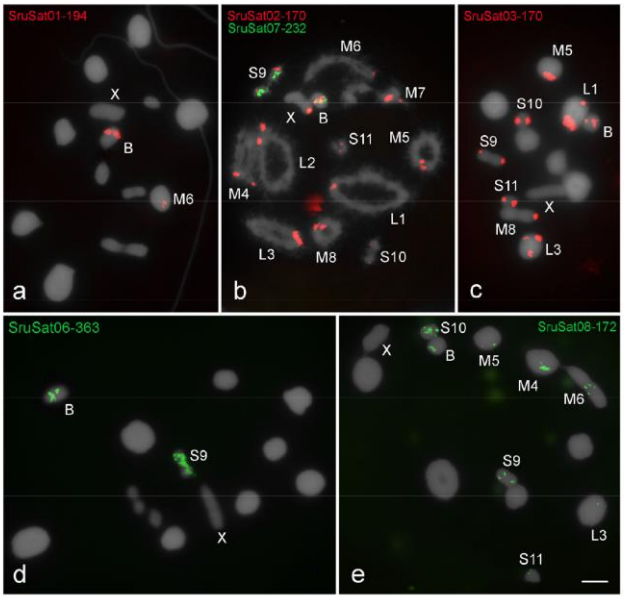

Figure 3. Chromosomal distribution of satDNAs in meiotic cells of *S. rubiginosa* (**a**,**c**,**d**,**e**) metaphase I and (**b**) diplotene. The distinct satDNAs families are indicated. Chromosomes with signals: X and B chromosomes are identified. Observe that the B chromosome harbors all satDNAs families, three of them shared with some A chromosomes (SruSat02-170, SruSat03-170, and SruSat08-172), and three exclusively shared with pair M6 (SruSat01-194) or pair S9 (SruSat06-363 and SruSat07-232). Bar = 5 μm.

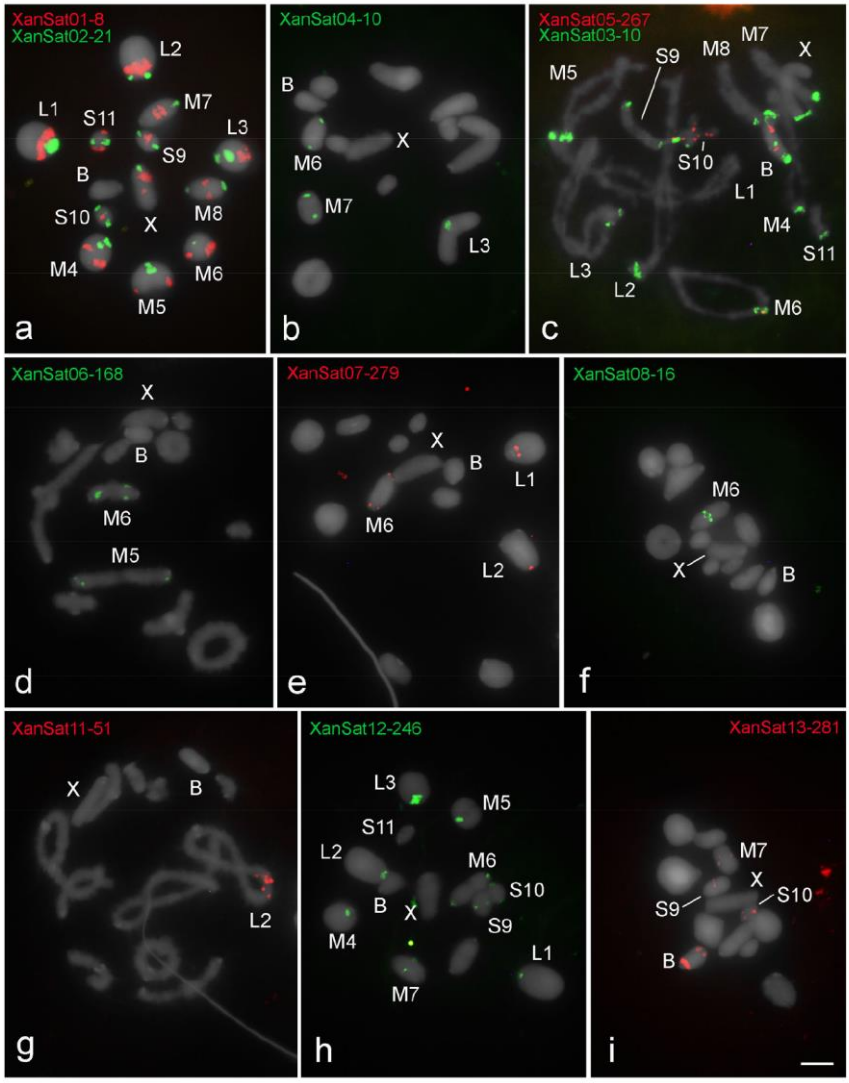

Figure 4. Patterns of chromosomal location revealed by FISH of eleven clustered satDNAs in *X. d. angulatus*. (**a**,**b**,**d**–**f**,**h**,**i**) metaphase I, (**c**,**g**) diplotene. The distinct satDNAs families are indicated. Chromosomes with signals: X and B chromosomes are identified. Note only three satDNA families on the B chromosome, XanSat03-10 (**c**), XanSat05-267 (**c**) and XanSat13-281 (**i**), all of which shared the pair S10. Multiply satDNA sites are observed for all satDNAs (**a**–**e**,**h**,**i**), except XanSat-08-16 (**f**) and XanSat11-51 (**g**). Bar = 5 μm.

The B chromosomes were enriched with distinct satDNA families. All of them were shared with the A chromosomes but show distinct patterns of distribution, such as occurrence in multiple chromosomes or occurrence restricted to one or few elements (Figures 2–5). Four satDNAs occupying pericentromeric regions were seen in the B chromosome of *R. brasiliensis*, RbrSat01-171 (Figure 2a), RbrSat03-36 (Figure 2b), RbrSat04-168 (Figure 2c), and RbrSat08-176 (Figure 2e). These satDNAs were shared with the chromosome S11, which accumulated them in the pericentromeric region. The satDNA RbrSat04-168 was exclusively shared between pair S11 and the B chromosome, while the others were also located in other chromosomes (Figure 2a–c,e and Figure 5a).

The B chromosome of *S. rubiginosa* harbored the six satDNAs that were found clustered in A chromosomes (Figures 3 and 5b). SruSat08-172 (Figure 3e) was located in the pericentromeric region, while the other satellites were interstitially located presenting differences in signal size (Figure 3a–d). The chromosome S9 harbored five of the six satDNAs present in the B chromosome, and among

them two were exclusive of pair S9 and B chromosome, SruSat06-363 (Figure 3d) and SruSat07-232 (Figure 3b). This was the chromosome with highest number of satDNAs shared with the B chromosome. SruSat01-194 was also in the B chromosome but among the A chromosomes this repeat was only in pair 6 (Figure 3a).

Among the eleven repeats mapped by FISH in *X. d. angulatus* chromosomes only three were visualized in the B chromosome, XanSat03-10 (Figure 4c), XanSat05-267 (Figure 4c), and XanSat13-281 (Figure 4i). For these repeats more than one signal was seen in the B chromosome (Figure 4c,i and Figure 5c). Xansat03-10 was located in pericentromeric, interstitial, and distal regions (Figures 4c and 5c), XanSat05-267 presented two interstitial blocks (Figures 4c and 5c) and XanSat13-281 was placed in interstitial and distal areas (Figures 4i and 5c). We observed that none of the satDNAs located on the B chromosome were restricted to one A chromosome, XanSat03-10 (Figures 4c and 5c) was located in all pericentromeric regions, XanSat05-267 (Figures 4c and 5c) was located in pairs M6 and S10, and XanSat13-281 (Figures 4i and 5c) was located in pairs M7, S9, and S10. Chromosome S10 shared the highest amount of satDNAs with the B chromosome (Figure 4c,i and Figure 5c).

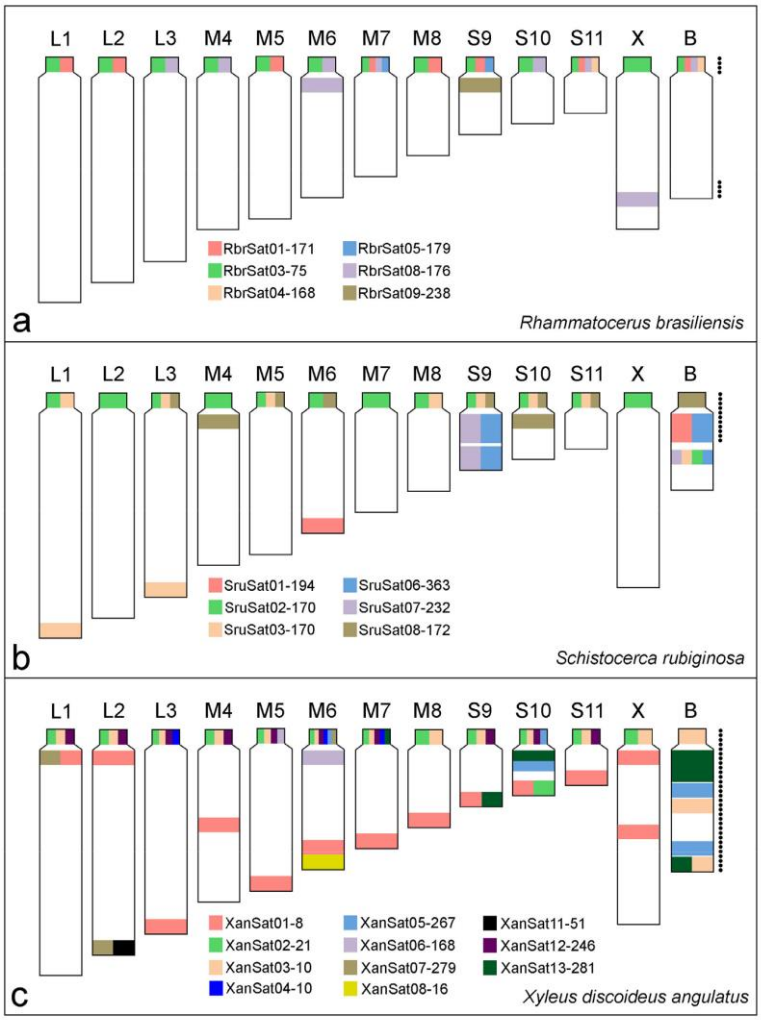

Figure 5. Ideograms summarizing the chromosomal location of clustered satDNAs in the three species of grasshoppers, (**a**) *R. brasiliensis*, (**b**) *S. rubiginosa*, and (**c**) *X. d. angulatus*. Each satDNA family is represented by one color. Black dots next to the B chromosomes indicate heterochromatin distribution in these chromosomes.

4. Discussion

Appling bioinformatic and molecular cytogenetic approaches to determine the content of satDNA allowed for a rapid increase in characterization of these kinds of elements among Orthoptera. High-throughput analysis has allowed the characterization of 234 satDNAs families in seven species [7,31,39–42]. Here we describe for first time the chromosomal organization of the most abundant satDNAs populating the genomes of three grasshopper species from the Romaleidae and Acrididae families. They correspond to a total 39 satDNAs families contributing to the knowledge of chromosomal organization of this kind of repeat on B chromosome.

SatDNAs Reveal Clues about B Chromosome Composition and Ancestry

Even though B chromosomes have been studied for a long time, we know very little about their ancestry in most species. B chromosome ancestry is known only in a few species [7], and therefore its origin still remains intriguing due in part to their high evolutionary rate. In grasshoppers, repetitive DNAs have been used to track B chromosome origin and composition in relatively few species. In *E. monticola* for instance, B chromosome ancestry is attributed to the autosomal pair S8 based on satDNAs analysis [7]. In *A. flavolineata*, the origin of B chromosome from pair 1 is attributed to the unique presence of U2 snDNA genes in these two chromosomes [16]. The ancestry of B chromosome in *L. migratoria* is related to pairs 8 and 9 due to the presence of satDNAs and histone genes in those chromosomes [8,15]. Previous works discussed the composition and putative origin of B chromosomes in two grasshoppers species studied here, *R. brasiliensis* and *X. d. angulatus*. However, it was not possible to elucidate a specific ancestral chromosome (see below). Based on satDNA content and organization we provide some clues about the ancestry of B chromosomes in these species, and additionally in *S. rubiginosa.*

The B chromosome of *R. brasiliensis* harbors four satDNAs, three of them are shared with multiple chromosomes (i.e., RbrSat01-171, RbrSat03-75, and RbrSat08-176) and, because of this, they are not good markers for ancestry determination. Most A chromosomes share two satDNAs with the B chromosome. The A chromosomes that share more satDNAs with the B chromosome are pairs M7 and S11, three and four, respectively, being good candidates to be involved in the origin of B chromosome. Three satDNAs (RbrSat01-171, RbrSat03-75, and RbrSat08-176) shared between pair M7, S11, and the B chromosome are also located on other A chromosomes. Furthermore, the pair S11 harbors the satDNA RbrSat04-168 that is an exclusive sequence shared with the B chromosome. The pericentromeric region of pair S11 fits exactly the composition of pericentromeric region of the B chromosome, supporting the hypothesis of its involvement in B chromosome origin.

Some controversial ideas were proposed for the origin of B in *R. brasiliensis.* First, the authors of a previous paper [35] proposed the origin from one or several chromosomes, including, for example, the pair S11 as supported here by satDNA mapping. Pairs L2, L3, M5, and S11 harbor 5S rDNA clusters that are shared with the B chromosome [35] and could be involved in its origin. Second, the analysis made by the authors of a previous paper [14] did not support the autosomal origin hypothesis, based on the presence of 5S rDNA and H3 gene clusters in the B chromosome that are shared with most A chromosomes (including the X chromosome), except the pair S11. However, the occurrence of these repetitive DNAs in the B chromosome could be more related to transposition events after its origin than ancestry [14]. These data, including the individuals from distinct populations, support the multiregional origin of the B chromosome in *R. brasiliensis* or dynamics for repetitive DNA organization for both A and B chromosomes, causing the emergence of new B chromosome variants. Based on a cytomolecular analysis, multiple B chromosome variants were described, for example, in rye, *Secale cereale* [43], and in the grasshoppers *X. d. angulatus* [36] and *E. plorans* [44].

Although our findings strongly support the ancestry of the B chromosome from the pair S11 in *R. brasiliensis* (at least in Lagoa do Carro/PE population), we should also point attention to the pair M7 that share three satDNAs with the B chromosome. In other populations (including Lagoa do Carro/PE) the chromosome M7 harbors H3 histone gene, which in some individuals is exclusively shared with the

B chromosome [14,45]. This suggests the involvement of M7, besides the pair S11, in B chromosome ancestry. It is similar to *L. migratoria* in which the B chromosome ancestry is putatively from two chromosomes, the pairs 8 and 9 [8], as in rye [46]. On the other hand, we should bear in mind that the pair M7 harbors one satDNA (RbrSat05-179) that is not observed in the B chromosome. Moreover, considering the high dynamism of the H3 histone gene (in number of clusters) in *R. brasiliensis*, it is possible that this gene was acquired later by the B chromosome. To shed light on this possibility, individuals from multiple populations should be studied using the distinct probes.

The satDNA mapping in *S. rubiginosa* suggests an autosomal origin for B chromosome from the pair S9. This chromosome shares five satDNAs with the B chromosome, two of them exclusive for this chromosome (SruSat06-363 and SruSat07-232), thus supporting the ancestry of the B chromosome from this bivalent. Furthermore, the pair S9 also harbors other three satDNAs present in the B chromosome, SruSat02-170, SruSat03-170, and SruSat08-172. Interestingly, those two exclusive satDNAs in S9 also are abundant in the B chromosome. This means that these repeats were massively amplified covering almost the entire length of those two chromosomes. The SruSat01-194 that is present in the pair M6 is also highly abundant in the B chromosome. We ruled out the possibility of B origin from M6 due to the absence of other three satDNAs that are present in the B chromosome, including those pericentromeric satDNA. Furthermore, if the pair M6 is involved in B chromosome origin it has a secondary contribution in comparison to the pair S9. It should be noted that SruSat01-194 corresponds to the most abundant satDNA in the *S. rubiginosa* genome, visible in the B chromosome as a large block likely due to amplification after its origin. It might be possible that the presence of this repeat in other A chromosomes (including pair S9), but arranged non-tandemly, makes it difficult to reach the FISH threshold resolution.

The satDNA content and distribution in the B chromosome of *X. d. angulatus* indicate a more complex evolution than in *R. brasiliensis* and *S. rubiginosa*, with additional chromosomal rearrangements after the B chromosome origin followed by accumulation/deletion involving repeats, as suggested by the previous analyses [35,36] (see below). Even though there are 11 satDNAs clustered on the A chromosomes of *X. d. angulatus*, only three of them are present in the B chromosome. There is no satDNA exclusively shared between the B chromosome and one chromosome of A complement. However, the pair S10 shares the most satDNAs with B chromosome (three satDNAs families), and it seems to be the ancestral pair involved in the B chromosome origin. The three satDNAs shared between B chromosome and the pair S10 are located at pericentromeric or interstitial regions (not far from the centromere), highlighting the origin of the B chromosome from about the half proximal part of the pair S10. Recently, it was suggested that pericentromeric and proximal regions enriched of repetitive DNAs were involved with the B chromosome origin in *X. d. angulatus*, followed by repetitive DNA amplification and rearrangements, like inversions [36].

Although the origin of the B chromosome in *X. d. angulatus* from the proximal part of the pair S10 is supported by current data, the presence of two other satDNAs in the pericentromeric region of pair S10 (XanSat02-21 and XanSat12-246), not shared with the B chromosome, is contrary to this hypothesis. The difference between the satDNA content in B and chromosome S10 can be explained by the changes of satDNAs amounts in the B chromosome during its evolution. In that way, the satDNA XanSat03-10 was amplified in the pericentromeric region of the B chromosome, while the other ones were completely deleted or conserved in small copy number, not detected by FISH. Interestingly, XanSat03-10 is a unique satDNA exclusively located in the pericentromeric region of all A chromosomes, likely involved in centromeric function. This could be the explanation for its amplification in the centromere of B chromosome, giving more stability through cell divisions. Besides amplification/deletion of satDNAs, the distribution of repeats in the B chromosome suggests the possibility of putative events of duplication and inversion that gave origin to the terminal region. The amplification of satDNAs after its origin and the changing satDNA repeat abundance was postulated in *E. monticola* [7]. Moreover, the putative duplication and inversion on the B chromosome

of *X. d. angulatus* highlights how dynamic the repetitive DNAs are on this element, leading to the emergence of distinct morphotypes.

5. Conclusions

The present data expands the knowledge about the B chromosomes composition and their origin in grasshoppers. Our results provide support for the intraspecific origin of the B chromosome in the three species, like in the other species of grasshoppers [7,16]. Although the B chromosomes share some meiotic peculiarities with the X chromosomes that suggested origin from this chromosome, the current knowledge indicates a more common origin from autosomes in grasshoppers [7,8,16,47]. Furthermore, the species studied here and other grasshopper species with B chromosome ancestry [7,8,47] support the recurrent involvement of small chromosomes in the B chromosome origin. This could be due to the fewer number of genes and the enrichment of repetitive DNAs in small autosomes [7,31,48]. The analysis of other populations employing the repetitive DNA markers used here will shed light on the evolution of B chromosome polymorphism in the species.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4425/9/11/523/s1, Table S1. Primers designed in this work and used for PCR amplification of satellite DNAs in the three species of grasshoppers.

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- In the referee suggestions field, up to five reviewer names can be entered by the author(s); valid e-mail contact addresses for these are required, in case they are selected by the editor. These suggestions can be made separately as preferred and not-preferredreviewer(s).
- Files must be uploaded separately and identified according to file types. The main text file must include references and, if applicaple, figure legends, which must be typed on a separate page following the References and Internet Resources sections. Each table, figure and element containing supplementary material must be saved and uploaded in a separate file. Formats for text and tables are Word or RTF in Windows platform. Figures should be in TIFF or JPEG formats (see detailed instructions in 3.1.h).
- Manuscripts including photos or any other identifiable data of human subjects must be accompanied by a copy of the signed consent by the individual or his/her guardian. Failure to adhere to these guidelines can delay the handling of your contribution and manuscripts may be returned before being reviewed. Special attention should be given to the structuring of the manuscript and correct language usage. These are important factors in the smooth running of the editorial and peer-review process, and can result in faster publication.

1. Categories of Contribution

Research Articles

Manuscripts must be written in English in double-spaced, 12-point type throughout; marked with consecutive line and page numbers, beginning with the cover page. The following elements must start on a new page and be ordered as they are listed below:

The title page must contain: a concise and informative title; the authors' names (first name at full length); the authors' institutional affiliation, including department, institution, city, state or province, and country; different affiliations indicated with superscript Arabic numbers; a short running title of up to 35 characters (including spaces); up to five key words; the corresponding author's name, full postal, and email address.

The Abstract must be a single paragraph that does not exceed 200 words and summarizes the main results and conclusions of the study.

The text must be as succinct as possible. Text citations: articles should be referred to by authors' surnames and date of publication; citations with two authors must include both names; in citations with three or more authors, name the first author and use *et al*. List two or more references in the same citation in chronological order, separated by semi-colons. When two or more works in a citation were published in the same year, list them alphabetically by the first author surname. For two or more works by the same author(s) in a citation, list them chronologically, with the years separated by commas. (Example: Freire-Maia *et al.*, 1966a, 1966b, 2000). Only articles that are published or in press should be cited. In the case of personal communications or unpublished results, all contributors must be listed by initials and last name (*et al*. should not be used). Numbers: In the text, numbers nine or less must be written out except as part of a date, a fraction or decimal, a percentage, or a unit of measurement. Use Arabic numerals for numbers larger than nine.Binomial Names: Latin names of genera, species and infraspecific taxa must be printed in italics; names of orders and families should appear in the Title and also when first mentioned in the text. URLs for programs, data or other sources should be listed in the Internet Resources Section, immediately following the References Section, not in the text.

The text includes the following elements:

Introduction - Description of the background that led to the study.

Material (or Subjects) and Methods - Details relevant to the conduct of the study. Statistical methods should be explained at the end of this section.

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Discussion - The findings of the study should be placed in context of relevant published data. Ideas presented in other publications should not be discussed solely to make an exhaustive presentation.

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The Acknowledgments must be a single paragraph that immediately follows the discussion and includes references to grant support.

The References Section: References must be ordered alphabetically by the first author surname; references with the same first author should be ordered as follows: first, as single author in chronological order; next, with only one more co-author in alphabetical order by the second author; and finally followed by references with more than two co-authors, in chronological order, independent of the second author surnames. In references with more than 10 authors only the first ten should be listed, followed by *et al*. Use standard abbreviations for journal titles as suggested by NCBI (http://www.ncbi.nlm.nih.gov/journals/

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g) Tables: must be in Word format prepared with the table tool (do not use space bar). A concise title should be provided above the table. Tables must be numbered consecutively in Arabic numerals. Each column must have a title in the box head. Footnotes typed directly below the table should be indicated in lowercase superscript letters. Tables that are to appear in the printed version must be saved in Word format and not as figures, so that they can later be fitted during typesetting. Each table must be saved and uploaded as a separate file.

h) Figures must be numbered consecutively using Arabic numerals. Images should be in TIFF or JPEG format. Figures in Word, PowerPoint or Excel format cannot be published. Only sequence data can be presented in Word format. Journal quality reproduction will require grayscale resolution yielding 300 dpi, color figures should be at 600 dpi. These resolutions refer to the output size of the file, that is the size in which it will appear printed in the journal; if it is anticipated that images will be enlarged or reduced, the resolutions should be adjusted accordingly. Figures composed of several elements should be sent as a single panel, obeying the print size definitions of the journal (single or two columns width). Scanned figures should not be submitted. Color illustrations are accepted. Each figure/panel must be saved and uploaded as a separate file. When uploading, identify each illustration by the first author name and the number of the respective figure. Figure legends must be included at the end of the main text file and should be typed on a new page.

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Supplementary material - the following online material is available for this article:
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3.2 Short Communications

Short Communications present brief observations that do not warrant full- length articles. They should not be considered preliminary communications;

- should be 15 or fewer typed pages in double spaced 12-point type, including literature cited;
- should include an Abstract;
- but no further subdivision, with introduction, material and methods, results and discussion; all in a single section and without headers.
- up to four items (tables and/or figures) may be submitted;

Note: The title page, abstract and reference section format is that of a full- length Research Article. For Supplementary Material see instructions in item 3.1.m.

3.3 Genome Insight For GMB

A new section of articles devoted to genome data (Genome Insight) will be considered for publication in Genetics and Molecular Biology. Genome Insight is for focused papers, usually of approximately 1500 words (up to four tables or figures), that publish new genome data as they become submitted to GenBank. This section is the premier forum to deliver that information directly to the genome community in a rapid and efficient publication of the genome. Data must be related to a complete (or nearly complete) and fully annotated genome for prokaryote or viruses, but a draft may be accepted for an eukaryote genome. While the focus of Genome Insight is necessarily involved in novel sequences, the manuscript must contain specifically novel biological, evolutive, biotechnological and/or metabolic insights revealed by data. The work may provide comparative analyses of previously published genomes that contain a substantial and novel insight of broadest biological and genetic significance. Submitted manuscript must contain an abstract, which should be a brief report on the organism as well as its relevance and the main insight revealed by the genome. The text (approximately 1500 words- excluding abstract, references and acknowledgements) should not contain subdivisions, but must contain the rational for the selection of such organism as well as organism information (including taxonomy, natural habitat, phylogenetic position, eventual pathogenicity, symbiotic, biotechnological use, etc), methodology (genome sequencing and assembly; reference number at GenBank), genome relevance (which should indicate the main insights revealed by the data analysis and main conclusions. Acknowledgements and References (up to 20 references) headings should be included. Figure Legends should be provided at the end of the manuscript. Metagenome, transcriptome as well as epigenome data may also be considered for publication, but prior submission of the abstract to the Editor is necessary. Note: The title page, abstract and reference section format is that of a full-length Research Article. For Supplementary Material see item 3.1.m. in our Instructions to Authors.

Letters to the Editor

Relate or respond to recent published items in the journal. Discussions of political, social and ethical issues of interest to geneticists are also welcome in this form.

Review Articles

Review Articles are welcome. The Editor must be contacted prior to submission. Please, provide an Abstract and a list of your recent publications in the area.

Book Reviews

Publishers are invited to submit books on Genetics, Evolution and related disciplines, for review in the journal. Aspiring reviewers may propose writing a review.

History, Story and Memories

These are accounts on historical aspects of Genetics relating to Brazil.

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Wrote the paper

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The criteria below are a guideline and are used also by editors and referees to assess manuscripts submitted to MGG. Hence it is advisable to make clear within the manuscript itself and/or in an accompanying letter how manuscripts meet such criteria.

Original Papers should describe and appropriately interpret results of a complete study. "Complete" in this sense does not refer to the length of an article. However, the results presented must justify all conclusions drawn and those conclusions should constitute a significant step ahead in the relevant field. The study presented must arrive at a defined endpoint, which ideally will stimulate further research. Generally, the results presented should be of interest well beyond the lab of the authors or should be likely to become of such wider interest in the foreseeable future. Hence, the manuscript must be prepared such that its content is accessible to a wide audience.
We welcome articles based on analysis of genome sequences leading to novel insight and/or stimulate further experimental research. We also welcome theoretical studies that stimulate future experimental studies.

Methodology Papers describe and document a new, or substantially improved method/approach/software/database, without using it to address a research question (in which case the paper would be an Original Paper). The method described should be of interest for a large number of laboratories.
Reviews should summarize the present knowledge in a certain field or topic, identify open research questions and/or phrase new and exciting hypotheses. Such reviews can be more comprehensive (up to 20 printed pages) or more concise but should be focused around an attractive question of research. The most important criteria are that reviews are of interest to a wider audience and/or make a wide audience aware of novel and interesting aspects. Authors are very welcome to critically discuss present knowledge and even to speculate, as

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Original Papers and Methodology Papers should be written using the following structure

Introduction
The introduction should guide the reader and hence contain the following:
A short introduction to the field of research
The scope and incentives of the work
The background, i.e. previous work by your and other research groups strictly on the subject
A conclusion summarizing the novelty and the significance of this paper.
Make sure that the introduction focuses on the background that is relevant for the data presented and the conclusions drawn.

Materials and Methods
This section should provide sufficient information to precisely evaluate how the data have been obtained, including the number of repetitions, and should permit a different research group to repeat the experimental work.
Results
Data should be presented as precisely and concisely as possible, if appropriate in the form of tables or figures. Make sure not to repeat in the text values given in figures or tables.
The data presentation should follow a logical flow starting with a short description of the incentive of the experiment, followed by a description of the results. Do not repeat information on the experimental details; those belong to Materials and Methods. Describe and discuss the data, but interpretations and conclusions should be given in the Discussion.
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The discussion should cover the following aspects:
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Any models realistically emerging from the data presented
Implications for future research
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The title page should include:

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Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

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Please provide 4 to 6 keywords which can be used for indexing purposes.

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Cite references in the text by name and year in parentheses. Some examples: Negotiation research spans many disciplines (Thompson 1990).
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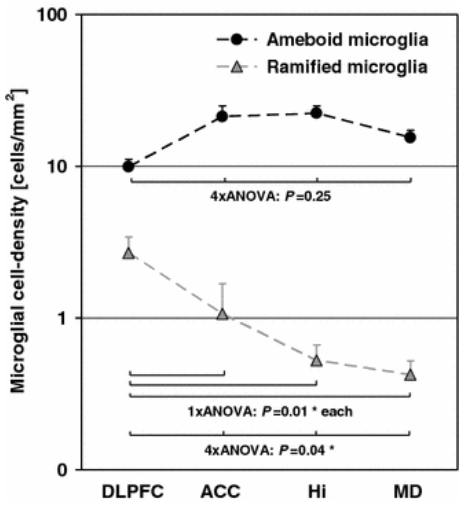

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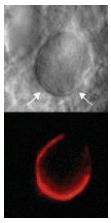

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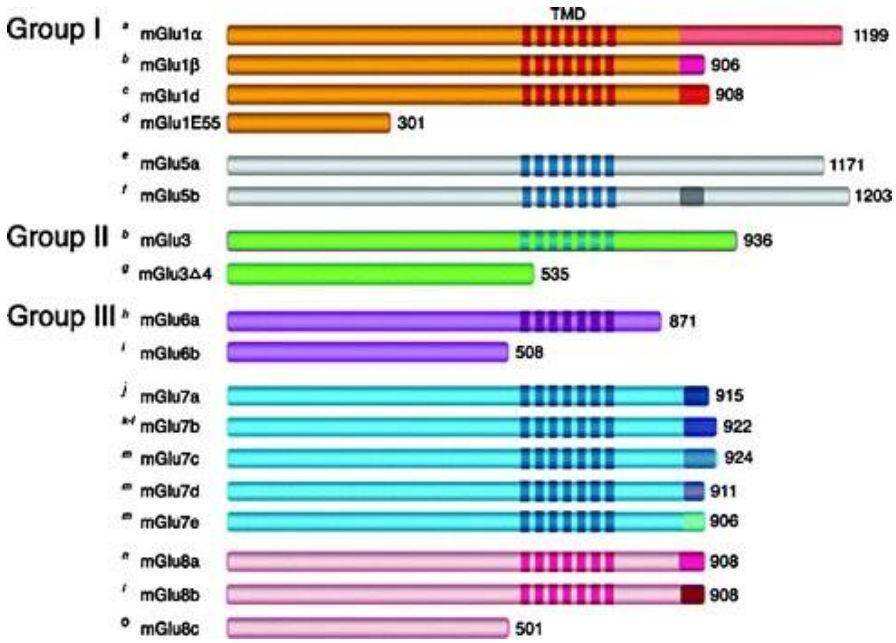

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Funding: This study was funded by X (grant number X).

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Adriana de Souza Melo

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Resumo informado pelo autor

Doutoranda em Genética pela Universidade Federal de Pernambuco (defesa agendada para março de 2019), possui mestrado em Biologia Celular e Molecular Aplicada pela Universidade de Pernambuco (2014) e graduação em Bacharelado em Ciências Biológicas pela Universidade de Pernambuco (2010). Tem experiência na área de Genética, com ênfase em Citogenética e biologia molecular.

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Formação acadêmica/titulação

2015 Doutorado em Genética.
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Título: Origem, dispersão e evolução do cromossomo B de Rhammatocerus brasiliensis (Orthoptera - Acrididae)
Orientador: Rita de Cássia de Moura
Bolsista do(a): Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco

2012 - 2014 Mestrado em Biologia Celular e Molecular Aplicada.
Universidade de Pernambuco, UPE, Recife, Brasil
Título: MAPEAMENTO CITOGENÉTICO DOS SÍTIOS DE HISTONA H3 e DNAr 5S EM Rhammatocerus pratensis (ORTHOPTERA - ACRIDIDAE) EM DIFERENTES POPULAÇÕES DO ESTADO DE PERNAMBUCO, Ano de obtenção: 2014
Orientador: Rita de Cássia de Moura
Co-orientador: Diogo Cavalcante Cabral de Mello
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico

2007 - 2010 Graduação em Bacharelado em Ciências Biológicas.
Universidade de Pernambuco, UPE, Recife, Brasil
Título: ANÁLISE DA HETEROCROMATINA CONSTITUTIVA EM SEIS ESPÉCIES DO GÊNERO Dichotomius (COLEOPTERA, SCARABAEIDAE)
Orientador: Diogo Cavalcante Cabral de Mello
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Formação complementar

2011 Elementary english. . (Carga horária: 170h).
Serviço Nacional de Aprendizagem Comercial, SENAC, Brasil

2014 - 2014 Intermediate English. . (Carga horária: 170h).
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2013 - 2013 Extensão universitária em Bioinformática: análise de dados moleculares. (Carga horária: 90h).
Universidade Federal de Pernambuco, UFPE, Recife, Brasil

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Serviço Nacional de Aprendizagem Comercial - PE, SENAC/PE, Recife, Brasil

2011 - 2011 Extensão universitária em Identificação de gêneros de Scarabaeinae do Brasil. (Carga horária: 2011h).
Universidade de Pernambuco, UPE, Recife, Brasil

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Serviço Nacional de Aprendizagem Comercial, SENAC, Brasil

2010 - 2010 Extensão universitária em Citogenética Molecular Animal.. (Carga horária: 40h).
Universidade de Pernambuco, UPE, Recife, Brasil

2009 - 2009 Curso de curta duração em Citogenética: da era clássica à molecular.. (Carga horária: 4h).
Sociedade Brasileira de Genética, SBG, Ribeirao Preto, Brasil

2009 - 2009 Extensão universitária em Curso de Genética Molecular Humana. (Carga horária: 60h).
Universidade de Pernambuco, UPE, Recife, Brasil

2009 - 2009 Extensão universitária em Avanços no Diagnóstico Mol. e Genética Humana. (Carga horária: 44h).
Universidade Federal de Pernambuco, UFPE, Recife, Brasil

2007 - 2007 Curso de curta duração em Minicurso de Histotécnica. (Carga horária: 20h).
Universidade de Pernambuco, UPE, Recife, Brasil

Atuação profissional

1. Vestibular Cidadão - VC

Vínculo institucional

2013 - 2013 Vínculo: Professor Visitante , Enquadramento funcional: Estudante , Carga horária: 2, Regime: Parcial
Outras informações:
Aulas Ministradas no Vestibular cidadão, Faculdade de Direito do Recife, UFPE. Carga horária total de 100 horas.

2. Universidade de Pernambuco - UPE

Vínculo institucional

2009 - 2010 Vínculo: Monitoria em Genética , Enquadramento funcional: Monitoria, Regime: Parcial

2008 - 2010 Vínculo: Estagiária , Enquadramento funcional: Estudante/estagiário , Carga horária: 20, Regime: Parcial

Áreas de atuação

1. Genética
2. Citogenética
3. Citogenética Animal
4. Citogenética de insetos

Prêmios e títulos

2012 Menção honrosa – Localização de sítios de DNAr 18S em duas espécies de Dynastinae (Coleoptera: Scarabaeidae) usando bandeamento NOR e hibridização in situ fluorescente., XIX Encontro de genética do Nordeste, I Simpósio de genética humana e médica do nordeste

2012 Menção honrosa - Análise da ocorrência e prevalência do cromossomo B no gafanhoto Rhammatocerus brasiliensis em diferentes populações de Pernambuco., Universidade de Pernambuco

Producão

Produção bibliográfica

Artigos completos publicados em periódicos

1. doi MILANI, DIOGO; BARDELLA, VANESSA; FERRETTI, ANA; PALACIOS-GIMENEZ, OCTAVIO; **MELO, ADRIANA**; MOURA, RITA; LORETO, VILMA; SONG, HOJUN; CABRAL-DE-MELLO, DIOGO
Satellite DNAs Unveil Clues about the Ancestry and Composition of B Chromosomes in Three Grasshopper Species. Genes. JCR, v.9, p.523 - , 2018.

2. doi AMORIM, IGOR COSTA; **MELO, ADRIANA DE SOUZA**; CRUZ, GEYNER ALVES DOS SANTOS; WALLAU, GABRIEL DA LUZ; MOURA, RITA DE CÁSSIA DE
Dichotomius (*Luederwaldtinia*) *schiffleri* (Coleoptera: Scarabaeidae) mitochondrial genome and phylogenetic relationships within the superfamily Scarabaeoidea. Mitochondrial DNA Part B. , v.2, p.887 - 888, 2017.

3. doi CABRAL-DE-MELLO, DIOGO CAVALCANTI; MOURA, RITA DE CÁSSIA; **SOUZA MELO, ADRIANA**; MARTINS, CESAR
Evolutionary dynamics of heterochromatin in the genome of Dichotomius beetles based on chromosomal analysis. Genetica ('s-Gravenhage). JCR, v.139, p.315 - 325, 2011.

Trabalhos publicados em anais de eventos (resumo)

1. **MELO, A. S.**; Cabral-de-Mello, DC
Evidências da origem do cromossomo B de Rhammatocerus brasiliensis (Orthoptera: Acrididae) In: XX Encontro de Genética do Nordeste, 2014, Campina Grande.
XX ENGENE. , 2014.

2. **MELO, A. S.**; Cabral-de-Mello, DC; MOURA, RITA DE CÁSSIA
Chromosomal mapping of the 5S rDNA and H3 histone gene in the grasshopper Rhammatocerus brasiliensis from distinct populations of the state of Pernambuco. In: 59º Congresso Brasileiro de Genética, 2013, Águas de Lindóia, SP.
. 59º Congresso Brasileiro de Genética. , 2013.

3. **MELO, A. S.**; MOURA, RITA DE CÁSSIA
Análise da ocorrência e prevalência do cromossomo B no gafanhoto Rhammatocerus brasiliensis em diferentes populações de Pernambuco In: Encontro de Pós-graduação Pesquisa e Extensão da Universidade de Pernambuco, 2012, Recife, PE.
Encontro de Pós-graduação Pesquisa e Extensão da Universidade de Pernambuco. , 2012.

4. FERREIRA NETO, C. A.; **MELO, A. S.**; MF Rocha; MOURA, RITA DE CÁSSIA
Localização de sítios de DNAr 18S em duas espécies de Dynastinae (Coleoptera: Scarabaeidae) usando bandeamento NOR e hibridização in situ fluorescente. In: XIX Encontro de genética do Nordeste, I Simpósio de genética humana e médica do nordeste, 2012, Petrolina, PE.
XIX Encontro de genética do Nordeste, I Simpósio de genética humana e médica do nordeste. , 2012.

5. FERREIRA NETO, C. A.; **MELO, A. S.**; MF Rocha; MOURA, RITA DE CÁSSIA
Localização de sítios de DNAr 18S em espécies de Cyclocephala (Coleoptera) usando AgNO3 e FISH. In: XV Congresso latinoamericano de genética/ XLI Congresso Argentino de genética/ XLIV Congreso de La sociedad de genética de Chile/ II Reunión regional SAG – Litoral, 2012, Rosário - Argentina.

XV Congresso latinoamericano de genética/ XLI Congresso Argentino de genética/ XLIV Congreso de La sociedad de genética de Chile/ II Reunión regional SAG – Litoral. , 2012.

6. FERREIRA NETO, C. A.; **MELO, A. S.**; MF Rocha; MOURA, RITA DE CÁSSIA
Caracterização citogenética clássica e molecular de Anomala violácea (Scarabaeidae, Rutelinae). In: XL Congreso Argentino de Genética/ III Simposio Latinoamericano de Citogenética y Evolución, 2011, Corrientes, Argentina.
XL Congreso Argentino de Genética/ III Simposio Latinoamericano de Citogenética y Evolución. , 2011.

7. Xavier, C; **MELO, A. S.**; FERREIRA NETO, C. A.; Cabral-de-Mello, DC; MOURA, RITA DE CÁSSIA
Estudo citogenético em Dichotomius schiffleri (Scarabaeidae): cariótipo, bandeamentos cromossômicos e localização do DNAr 18S. In: XL Congreso Argentino de Genética/ III Simposio Latinoamericano de Citogenética y Evolución, 2011, Corrientes, Argentina.
XL Congreso Argentino de Genética/ III Simposio Latinoamericano de Citogenética y Evolución. , 2011.

8. Cabral-de-Mello, DC; MELO, A. S.; Rita Moura; Martins,C
Repeated DNAs profile in six Dichotomius (Coleoptera, Scarabaeidae) species: heterochromatin and multigene family organization In: 56º Congresso Brasileiro de Genética, 2010, Guarujá.
56º Congresso Brasileiro de Genética. , 2010.

9. MELO, A. S.; Cabral-de-Mello, DC; Martins,C; Rita Moura
Análise citogenética de Dichotomius bos (coleoptera, Scarabaeidae): cariótipo e heterocromatina constitutiva In: 1° Reunião Brasileira de Citogenética, 2009, Águas de lindóia.
1° Reunião Brasileira de Citogenética. , 2009.

Apresentação de trabalho e palestra

1. **MELO, A. S.**; MOURA, RITA DE CÁSSIA
Origem, dispersão e evolução do cromossomo B de Rhammatocerus brasiliensis (Orthoptera - Acrididae), 2018. (Conferência ou palestra,Apresentação de Trabalho)

2. **MELO, A. S.**; Cabral-de-Mello, DC; Rita Moura
Evidências do sítio de origem dos genes de Histona H3 e DNAr 5S em Rhammatocerus brasiliensis (Orthoptera - Acrididae)., 2017. (Congresso,Apresentação de Trabalho)

3. **MELO, A. S.**; AMORIM, I. C.; WALLAU, G. L.; MOURA, R. C.
Organização do genoma mitocondrial de Rhammatocerus brasiliensis (Orthoptera - Acrididae) e análise filogenômica., 2016. (Congresso,Apresentação de Trabalho)

Eventos

Eventos

Participação em eventos

1. **59º Congresso Brasileiro de Genética**, 2013. (Congresso)

2. **XV Congresso latinoamericano de genética/ XLI Congresso Argentino de genética/ XLIV Congreso de La sociedad de genética de Chile/ II Reunión regional SAG – Litoral**, 2012. (Congresso)

3. **56º Congresso Brasileiro de Genética**, 2010. (Congresso)

4. **56º Congresso Brasileiro de Genética**, 2010. (Congresso)

5. **1ª Reunião brasileira de citogenética**, 2009. (Congresso)

6. **1° Reunião Brasileira de Citogenética**, 2009. (Outra)

7. **55º Congresso Brasileiro de Genética**, 2009. (Congresso)

8. **Atividades da Semana Universitária do ICB/UPE**, 2008. (Encontro)

9. **III Workshop de Genetica e Biologia Molecular de Insetos Vetores de Doenças Tropicais**, 2008. (Encontro)

10. **VIII Semana do Biólogo-Todo dia é Dia do Biólogo**, 2008. (Encontro)

11. **2º Simpósio em Ciências Fisiológicas Promovido pelo Departamento de Ciências Fisiológicas do ICB/UPE-PE**, 2007. (Simpósio)

12. **Atividades da Setima Semana Universitária no ICB/UPE**, 2007. (Encontro)

13. **Comemoração do Dia do Biólogo-Um Olhar Para o Mundo**, 2007. (Encontro)

14. **I Encontro de Pós-Graduação em Pesquisa e XII Seminário de Iniciação Científica/UPE-PEI**, 2007. (Encontro)

15. **Trilha dos Manguezais**, 2007. (Outra)

Organização de evento

1. **MELO, A. S.**
Encontro de Pós-Graduação e Pesquisa, 2014. (Outro, Organização de evento)

2. **MELO, A. S.**
Encontro de Pós-Graduação, Pesquisa e Extensão da Universidade de Pernambuco., 2012. (Outro, Organização de evento)

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