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RESEARCH ARTICLE

Variability of Minisatellite Loci and mtDNA in Individuals with and without B Chromosomes from Populations of the Grasshopper *Dichroplus elongatus*

N. Rosetti[1] · **M. I. Remis**[1]

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Abstract *Dichroplus elongatus* is an extensively distributed South American grasshopper considered a pest of major crops. Argentinean populations show a widespread B-chromosome polymorphism which could be maintained as the result trade-offs among opposite selective effects and interactions with their mitotic instability. The main objective of this study was to evaluate the relationships between B chromosomes and mtDNA sequences coupled with minisatellites loci, and verify the genotype/karyotype covariation in 12 populations located at both sides of Paraná River (Eastern and Western Regions). B carrier individuals showed significantly higher genetic diversity (H_E and X) respect to standard individuals. AMOVAs based on nuclear loci and mtDNA sequence datasets showed statistically significant levels of differentiation among karyotypes in the Eastern Region. Cluster analysis through Bayesian procedure considering nuclear loci splits B carriers and standard individuals into different genetic clusters in some Eastern populations. The Bayesian phylogenetic analysis showed two divergent mtDNA clades. Haplogroup 1 is composed exclusively of standard individuals, however all B chromosome carriers are included in haplogroup 2. There is an association between some haplotypes and B chromosomes and a strong effect of phylogenetic signal on B chromosome population structure. Genetic differentiation between karyotypes at Eastern Region revealed by AMOVA, Bayesian approaches and clustering analysis based on uniparental and biparental inherited markers may be due to the inherent nature of the B chromosome, to karyotype biased dispersal or to difference tolerance of B chromosomes on different genetic background. The combination of molecular and chromosome analysis performed in this study indicated that B chromosomes in *D. elongatus* is an important factor in explaining the genetic population structure at minisatellite and mitochondrial DNA levels.

Keywords Population genetic · Grasshopper · Minisatellite loci · MtDNA

✉ M. I. Remis
mariar@ege.fcen.uba.ar

[1] Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C1428EHA Buenos Aires, Argentina

Introduction

Heritable variation provides the raw material for future evolutionary change; therefore, the presence of different levels of variation in different populations provides evidence of different evolutionary and historic events (Timmermans et al. 2005).

Among insects, populations of the same species may sometimes exhibit a great amount of karyotype variation (Collinge et al. 2006). Insect chromosomal polymorphism has frequently been associated with environmental adaptation (Simard et al. 2009; Rosetti and Remis 2012).

Important insights into the process of intraspecific differentiation may be obtained by comparing chromosomal variation with molecular markers (Hoffmann et al. 2004). The simultaneous analyses of uniparental and biparental inheritance marker variation provide complementary information about the demography events and evolutionary processes that moulding genetic variation and constitute appropriate and robust approaches to identify genetically isolated evolutionary units.

Studies using mitochondrial DNA (mtDNA), a matrilineally inherited haploid marker, are often used to obtain

information related to female mediated gene flow and phylogeography in animals (Ortego et al. 2009; Pimper et al. 2010). Phylogeography analyses of genealogical relationships among lineages and geographic distribution, offering information about the demographic history of populations (Avise 2000). Standing approaches, based on coalescent theory, provide useful algorithms to infer historical demographic events (e.g. bottleneck, population expansion, range expansion) which may contribute meaningfully to the current genetic structure of a population (Tajima 1989; Slatkin and Hudson 1991; Harpending 1994).

Highly variable nuclear markers are valuable markers to infer population genetic structure and effective migration among local populations. Current genetic approaches based on maximum likelihood, Bayesian probability theory and Monte Carlo Markov chain simulation complemented traditional methods improving population genetic studies (Selkoe and Toonen 2006; Blanchet et al. 2012; Manrique-Poyato et al. 2013). Bayesian inferences use genetic information to assign individuals to populations without assuming predefined populations and landscape genetic analysis can to analyse the existence of probable landscape heterogeneity on gene flow and hence on genetic variation (Pritchard et al. 2000; Manel et al. 2003).

Simultaneous analysis of chromosome variation, genetic population structure and phylogeography patterns, provide an opportunity to explore the potential influence of chromosomal change in microevolutionary processes. Approaches incorporating molecular tools into analysis of chromosomal variation may clarify population processes that lead the maintenance of chromosome polymorphism in some environments (Kennington and Hoffmann 2013).

Many species of Orthoptera, especially those belonging to the family Acrididae, are considered opportunistic species and constitute an economic interest group. Besides the economic interest, the grasshoppers are a group of evolutionary interest because they offer certain advantages to detect describe and quantify microevolutionary processes due to most species has wide geographical distribution, no overlapping generations and large population sizes.

Species belonging to the genus *Dichroplus* spp. (Orthoptera: Acrididae) provide valuable biological models for studying levels and geographical structuring in the genetic variation in grasshopper pests. *Dichroplus elongatus* is a South American grasshopper, considered of great agroeconomic interest due to the damage caused in grasslands and cultivated areas. The wide geographic distribution of this species throughout Argentina, Uruguay, most of Chile and southern Brazil (Lange et al. 2005), and its apparent ubiquity (De Wysiecki et al. 1997), seem to have allowed it to benefit from the development of disturbed environments associated with agroecosystems (De Wysiecki et al. 2004; Lange et al. 2005). Previous studies indicated also the evolutionary interest of *D. elongatus* by demonstrating the existence of B chromosome polymorphisms (Remis and Vilardi 1986). B chromosomes are supernumerary chromosomes that frequently show accumulation mechanisms, do not recombine with the standard complement (A chromosomes) and follow their own evolutionary pathway (Beukeboom 1994; Camacho et al. 2000). Intrapopulation analysis demonstrated that *D. elongatus* showed a considerable phenotypic variation related to karyotype and some components of selection (Clemente et al. 1994; Rosetti et al. 2007, 2008; Rosetti and Remis 2013).

Adaptive environmental clines where chromosomal polymorphisms and morphometric traits running in parallel suggest that both might be related and subject to similar evolutionary forces (Orengo and Prevosti 2002). Natural populations of *D. elongatus* have recurrently shown clinal variation in B chromosome frequency and morphometric traits along latitudinal gradients. The observed pattern of phenotypic variation is likely to be the result of local adaptation to season length along the latitudinal gradient (Rosetti and Remis 2013).

Recent intraspecific diversity analysis in this grasshopper based on minisatellites markers (DAMD; Direct Amplification of Minisatellite Regions) and mtDNA sequences were examined (Rosetti and Remis 2012). Analysis on nuclear DAMD loci in populations distributed at both size of the Paraná River showed a significant genetic differentiation among populations based on the results of AMOVA and Bayesian cluster approaches whereas landscape analysis detect genetic discontinuities associated with environmental heterogeneity reflecting the changing agroecosystem mainly in Western Region with respect to Eastern Region (Rosetti and Remis 2012). Similarly, phylogeographic approaches revealed the existence of clear genetic differentiation between two groups of populations located at both margins of the Paraná River which became separated during climate oscillations of the Middle Pleistocene, suggesting a significant restriction in effective dispersion mediated by females and a significant role of the River as a geographical barrier (Rosetti and Remis 2012).

The main goal of the present work was to combine the karyotype results with uniparental and biparental inheritance marker datasets in order to cast some light about the probable chromosome-genetic background relationships. More specifically we aimed to analyse in B chromosome polymorphic population: (i) the degree of mitochondrial and minisatellite variation in relation to karyotype structure, and (ii) any possible association between karyotype with distinct mtDNA haplotypes and/or minisatellite loci.

Fig. 1 Sampling sites of 12 B chromosome polymorphic populations of *Dichroplus elongatus* separated by the Paraná River. Abbreviations correspond with Table 1

Materials and Methods

Sample Material

A total of 12 populations of *Dichroplus elongatus* separated by a natural barrier, the Paraná River, from Argentina were considered in this study. Distribution of sampling localities is shown in Fig. 1 and Table 1. We assessed populations which were previously analysed at molecular level using minisatellite loci (DAMD, Direct Amplification of Minisatellite Regions), and a region of 527 bases of the mitochondrial cytochrome oxidase I (COI) gen and were recently examined at chromosome level (Rosetti and Remis 2012, 2013).

Statistical Analyses

DAMD Analyses

DAMD variation could be analysed for different karyotypes in ten populations (YAP, MCA, MOC, GUA, CAR, CAÑ, RAU, FLO, RCO, HCM) (Fig. 1; Table 1). Amplified fragments per individual were scored by the presence (1) or absence (0) of every band in all individuals. Only reproducible bands were taken into account to generate the DAMD matrix dataset.

The expected heterozygosity (H_E) between B carriers and standard individuals within each polymorphic population, as well as the total H_E summarizing all individuals by karyotype without considering the population of origin, was estimated using AFLPsurv software package (Vekemans et al. 2002), following Lynch and Milligan (1994). We estimated allelic frequencies at DAMD loci using the Bayesian method developed by Zhivotovsky (1999) for diploid species. A nonuniform prior

Table 1 Geographic variables of 12 B chromosome polymorphic natural populations of *D. elongatus* located in Argentina

	Population	Latitude	Longitude	Altitude (m)
Western region	Raco (RCO)	26°36′S	65°10′W	1172
	Horco Molle (HCM)	26°48′S	65°19′W	550
	Campana (CAM)	33°59′S	58°57′W	20
	Carmen de Areco (CAR)	34°49′S	59°50′W	5
	Cañuelas (CAÑ)	35°03′S	58°46′W	28
	Las Flores (FLO)	35°55′S	59°07′W	38
	Rauch (RAU)	36°47′S	59°06′W	20
Eastern region	Colón (COE)	32°13′S	58°09′W	40
	Gualeguaychú (GUA)	33°06′S	58°32′W	5
	Mocoretá (MOC)	30°38′S	57°58′W	60
	Monte Caseros (MCA)	30°17′S	57°38′W	60
	Yapeyú (YAP)	29°28’S	56°49′W	40

distribution of allelic frequencies was assumed with its parameters being derived from the observed distribution of fragment frequencies among loci (Zhivotovsky 1999).

In addition, we estimated the number of bands (X) between B carriers and standard individuals within populations and grouping total individuals according to their karyotype, regardless their source population.

The differences in the number of bands between B carrier and standard (ST) individuals were analyzed by means of two way analysis of variance (ANOVA) considering karyotype and population as independent factors. Because H_E values did not show normal distribution, H_E comparisons between karyotypes were evaluated for significance using the Mann–Whitney U test. Both analyses were implemented using STATISTICA (STATISTICS STATSOFT Inc. 1996).

Divergence among the polymorphic sampled populations was assessed by a hierarchical analysis of molecular variance (AMOVA, Excoffier et al. 1992) using GENALEX software package (Peakall and Smouse 2001). Due to the dominant expression of DAMD markers, the AMOVA analyses partitioned variation according to correlations among genotypes rather than variation in gene frequencies.

Nonmetric multidimensional scaling (MDS) based on Nei genetic distances was used to assess the genetic structure in multidimensional space using STATISTICA (STATISTICS STATSOFT Inc. 1996).

Genetic differentiation between pairs of populations was analysed through pairwise F_{ST} comparisons. The statistical significance of the variance components of the AMOVA and the paired comparisons were determined by nonparametric procedures using 1000 random permutations. We also estimated genetic differentiation implementing a Bayesian approach for dominant markers without assuming Hardy–Weinberg by means of HICKORY software v. 1.0.4 (Holsinger et al. 2002). Results were examined under three probable models: (i) full model ($f \neq 0$ and $\theta^B \neq 0$); (ii) $f = 0$ model ($f = 0$ and $\theta^B \neq 0$); (iii) theta = 0 model ($f \neq 0$ and $\theta^B = 0$). The estimates of θ^B and f are the Bayesian equivalents of F_{ST} and F_{IS} Wright indices calculated using Markov chain Monte- Carlo matrices simulations. Comparisons of models were performed using the Deviant Information Criterion (DIC).

In order to analyse the degree of genetic structure according to the karyotype, all the genotypes were screened using a Bayesian admixture procedure implemented in STRUCTURE 2.1 (Pritchard et al. 2000). STRUCTURE was run with the admixture model and five repetitions of 150.000 iterations, following a burn-in period of 50.000 iterations. We assessed population structure, separating B carriers of ST individuals of each population.

mtDNA Sequence Analyses

Variation at mtDNA level and karyotype can be simultaneously analysed in ten populations (YAP, MCA, MOC, COE, GUA, CAR, CAM, CAÑ, FLO, RAU) (Table 1; Fig. 1). All sequencing was performed in both directions and the haplotype of each individual was verified on the basis of both sequences. Sequences were aligned using CLUSTAL 1.81 (Jeanmougin et al. 1998) and edited using BIOEDIT version 7.0.9 (Hall 1999).

Genetic variation among karyotypes was estimated using haplotype diversity h (Nei 1987) and nucleotide diversity π (Nei and Jin 1989) with the software ARLEQUIN version 3.5 (Excoffier et al. 2009).

The genetic structure of polymorphic populations was examined with an analysis of molecular variance (AMOVA) as implemented in the software ARLEQUIN 3.5 (Excoffier et al. 2009). A hierarchical AMOVA was achieved to test differences among regions (East and West sides of Parana River) and among karyotypes within regions. Furthermore, we conducted two individual AMOVAs taking into account the differences in karyotype within each region. Statistical significance of derived indexes (Φ_{ST}) was assayed through a nonparametric permutation method (5000 permutations). We used a Bayesian Inference method implemented in Mr BAYES 3.0 software (Huelsenbeck and Ronquist 2001) to reconstruct phylogenetic relationships among haplotypes. We used a general time reversible model with invariable sites and gamma distribution (GTR + C + I). Searches were run for 200,000 generations, sampling every 100 generations, with branch lengths recorded. After completion of the analysis, the first 50,000 trees were discarded as burn-in before a majority-rule consensus tree with posterior probabilities for each bipartition was calculated. Two searches were performed to confirm stationarity.

Results

To characterize genetic molecular variation in individuals with and without B chromosomes (B carrier and standard individuals), we considered variation for nuclear and mitochondrial markers previously examined in twelve polymorphic Argentinean populations of the grasshopper *D. elongatus* (Rosetti and Remis 2012, 2013). Populations were considered according to their geographic position respect of either side of Paraná River (Eastern and Western Regions) (Table 1; Fig. 1).

Direct amplified minisatellites DNA (DAMD) and chromosome variation was simultaneously examined in ten out of 12 sampled populations (YAP, MCA, MOC, GUA, CAR, CAÑ, RAU, FLO, RCO, and HCM). No diagnostic neither private bands were detected in B carrier individuals.

Table 2 DAMD genetic diversity indices of B carriers (B) and standard (ST) individuals, in 10 natural populations of *D. elongatus*

Population	N	H_E	SE (H_E)	X	SE (x)
CAR	17	0.410	0.009	81.176	1.596
CAR-B	3	0.450	0.009	92.667	3.799
RAU	18	0.421	0.007	81.556	1.551
RAU-B	3	0.452	0.010	86.667	3.799
FLO	20	0.418	0.009	87.100	1.471
FLO-B	3	0.423	0.010	93.333	3.799
CAÑ	17	0.399	0.008	73.706	1.596
CAÑ-B	2	0.361	0.013	84.500	4.653
MOC	17	0.412	0.008	85.058	1.596
MOC-B	3	0.439	0.010	93.667	3.799
GUA	17	0.407	0.008	82.294	1.596
GUA-B	3	0.447	0.010	88.667	3.799
MCA	20	0.433	0.006	88.350	1.471
MCA-B	2	0.450	0.009	92.500	4.653
YAP	19	0.420	0.007	81.789	1.509
YAP-B	2	0.434	0.009	99.500	4.653
RCO	16	0.441	0.007	87.375	1.645
RCO-B	7	0.455	0.006	88.571	2.487
HCM	17	0.451	0.006	96.353	1.596
HCM-B	6	0.473	0.006	93	2.686
ST	178	0.427	0.005	84.522	0.617
B	34	0.452	0.005	91.059	1.412

Mean expected heterozygosity (HE) and mean number of bands (X) with their standard error (SE) are shown

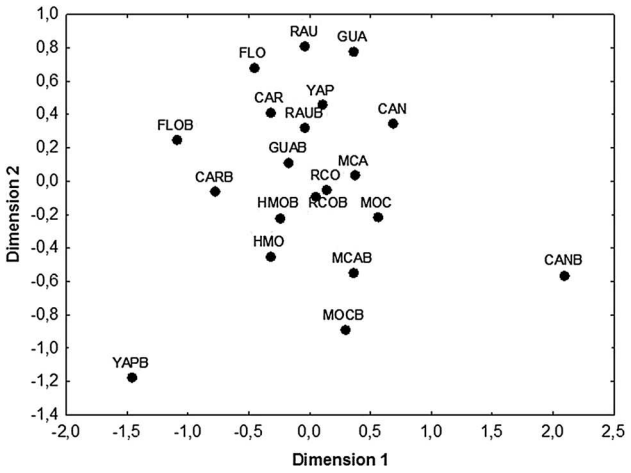

Fig. 2 Multi-dimensional scaling analysis based on Nei genetic distances and Euclidean chromosome distances for 10 populations of *Dichroplus elongatus*, separating B carriers from standard individuals in each population

Nuclear diversity estimates were calculated separately for each karyotype within every population and for the total sampled individuals regardless population of origin (Table 2). Mean expected heterozygosis (H_E) and mean number of bands (X) varied between karyotypes. As a general feature B carriers showed higher values of H_E and X with respect to standard individuals within each population. Two way ANOVA showed highly significant differences in the mean number of bands between karyotypes ($F_{1;117}$ =11.76; P=0.0008) and among populations ($F_{5;117}$=8.02; $P<10^{-4}$) in Western Region whereas significant differences between karyotypes are observed in Eastern Region ($F_{1;75}$=14.42; P=0.0003).

Additionally Mann–Whitney U Test showed significant differences in mean He estimates between B carriers and standard individuals in Eastern Region (Z_{HE} =−2.31; P=0.021). The multidimensional scaling analysis (MDS) based on Nei genetic distances showed that B-carriers frequently are more heterogeneous and differentiated from standard individuals (Fig. 2).

The hierarchical analysis of molecular variance (AMOVA) indicated that in the Eastern Region most of DAMD variation was found among individuals within karyotypes (89%) (F_{ST} = 0.108; P=0.001), whereas 8% of the variation was detected among populations (F_{CT} = 0.077; P=0.001) and 3% of variation could be attributed to differences between karyotypes within populations (F_{SC} = 0.034; P=0.0018). This analysis may indicate some genetic heterogeneity in minisatellite loci between karyotypes in populations located at the Eastern Region of Paraná River.

The hierarchical AMOVA in the Western Region demonstrated that 11% of DAMD variation can be identified among populations (F_{CT} = 0.11; P=0.001) whereas 89% of the variation can be attributed to differences among individuals within karyotypes (F_{ST} = 0.111; P=0.001). No significant DAMD variation was detected between karyotypes in populations belonging to Western Region.

The Bayesian analysis of population differentiation for the total data showed that the model that best fits the data is the full model (f and $\theta^B \neq 0$) as it has the lowest DIC value, both in Eastern and Western Regions (Table 3). However the DIC value for the f=0 model was slightly higher than the DIC value for the full model suggesting that only slight inbreeding occurred in these populations.

Bayesian approaches to detect karyotype differentiation revealed that the model that best fits the data is the full model for the Eastern Region demonstrating significant differences among karyotypes; in the Western Region in turn the DIC values obtained for the full model was very similar to those detected for the other two models showing weak genetic differentiation among individuals of different karyotypes (Table 3).

The clustering method implemented to check the concordance between karyotype and genetic structure, indicated that the most likely number of genetically distinct clusters was K=8 (lower value of log of marginal

Table 3 Estimates of DAMD differentiation (θB) through the Bayesian approach implemented in Hickory software including the Deviant Information Criterion (DIC) (95% credibility intervals)

Western Region	Full Model		f = 0 Model		$\theta^B = 0$ Model	
	θ^B	DIC	θ^B	DIC	θ^B	DIC
Among populations	0.099 (0.081–0.116)	4203.92	0.070 (0.063–0.081)	4220.51	–	4952.15
Among karyotypes within regions	0.017 (0.005–0.035)	1573.52	0.001 (0.0001–0.009)	1588.87	–	1588.87
Among populations	0.103 (0.083–0.117)	6707.78	0.073 (0.058–0.084)	6743.34	–	7937.29
Among karyotypes within regions	0.024 (0.016–0.035)	1834.75	0.017 (0.010–0.024)	1837.98	–	1918.34
Total	0.008 (0.003–0.014)	1936.69	0.006 (0.003–0.011)	1937.56	–	1961.49

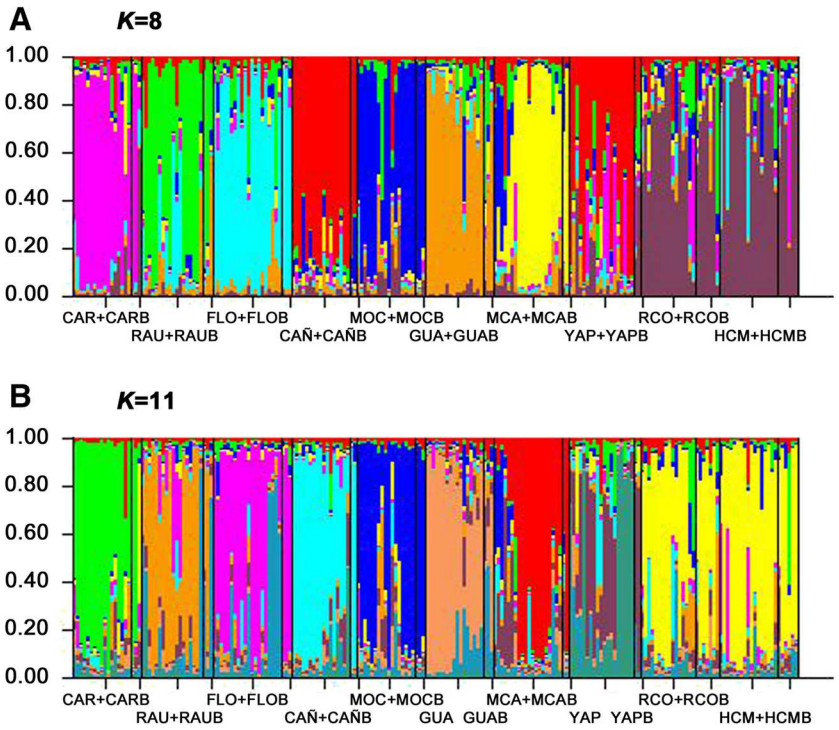

Fig. 3 Assignment probabilities analysis of individuals to genetic clusters considering karyotype, assuming correlated frequencies and admixed origin of 10 populations of *Dichroplus elongatus* for K = 8 (**A**) and K = 11 (**B**)

likelihood, −19507.2), because B carriers did not differ genetically from ST individuals within each population; besides, RCO + HCM were grouped as a single cluster, as well as YAP + CAÑ (Fig. 3 A). The second best value of log of marginal likelihood (−19541.03) was for K = 11, showing RCO + HCM as one cluster whereas YAP and YAPB fall into two separate genetic clusters and CAÑ and YAP are no longer grouped together (Fig. 3B); this suggests that the second best clustering, separates B carriers from ST individuals in YAP, instead of grouping YAP and CAÑ together. Furthermore GUA and GUAB also fall into two separate genetic groups indicating some degree of genetic differentiation between B carriers and standard individuals within this population.

Simultaneous analysis of variation in a fragment of the mitochondrial COI gene and karyotype was attained in ten out of twelve sampled populations (YAP, MCA, MOC, COE, GUA, CAR, CAM, CAÑ, FLO, RAU) (Table 1; Fig. 1). Only one mtDNA haplotype was observed in each individual, including B carriers, indicating an absence of heteroplasmy in the analysed samples. This result indicated that the PCR products obtained were the result of PCR amplification of mitochondrial genome suggesting that the B chromosome of *D. elongatus* does not carry COI mtDNA sequences. Haplotype frequencies as well as diversity indexes were included in Table 4.

Of the 32 original haplotypes identified in this species by Rosetti and Remis, (2012); 25 haplotypes were found in chromosomally polymorphic populations analysed here. One of the most common haplotype (DE-14) was detected in both karyotypes but the other frequent haplotype (DE-24) was detected only in standard individuals. B carriers located West of the Paraná River exhibited mainly similar haplotype distribution with respect to

Table 4 Summary of haplotype frequency and genetic diversity indices (gene diversity (h) with their standard deviation and nucleotide diversity (π) with their standard deviation) of B carriers and standard individuals located at Eastern and Western Regions of the Paraná River

Haplotype	East ST	East B	West ST	West B
DE-02	–	–	1	–
DE-04	–	–	1	–
DE-06	–	–	1	–
DE-07	1	–	–	–
DE-08	1	–	1	–
DE-09	–	–	2	–
DE-10	1	–	–	–
DE-11	1	–	–	–
DE-12	–	–	2	–
DE-13	3	1	–	–
DE-14	10	1	36	5
DE-15	–	–	2	–
DE-16	–	–	1	–
DE-17	2	–	–	–
DE-18	1	–	–	–
DE-19	–	–	–	1
DE-22	6	1	-	–
DE-24	8	–	2	–
DE-25	2	–	–	–
DE-26	4	5	–	–
DE-27	–	–	2	–
DE-28	–	–	1	–
DE-29	–	–	–	1
DE-30	1	–	–	–
N	41	8	52	7
h	0.879 (0.027)	0.643 (0.184)	0.539 (0.083)	0.551 (0.208)
π	0.011 (0.006)	0.013 (0.008)	0.007 (0.004)	0.008 (0.005)

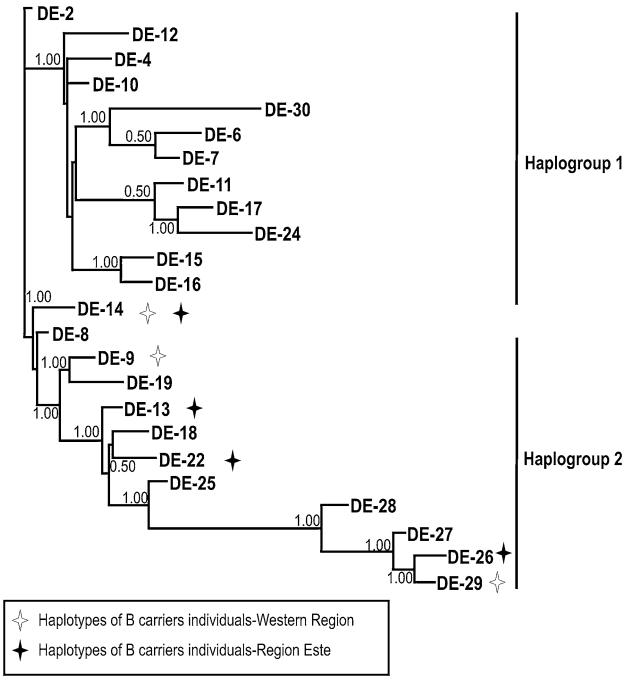

Fig. 4 Consensus tree from Bayesian phylogenetic analysis using a 527 bp mitochondrial COI fragment. *Internal nodes* show support as posterior probability

standard individuals. Individuals with B chromosomes at the Western Region showed largely the most frequent haplotype (DE-14) with some other rare haplotypes (DE-19 and DE-29). On the contrary, the B carriers at East of Paraná River showed high frequency (62.5%) of the forth most common haplotype (DE-26) and a similar frequency (12.5%) of other three haplotypes (DE-13, DE-14 and DE-22). B and standard individuals belonging to populations located at East of Paraná River shared four haplotypes of the 13 identified in the region, whereas individuals with different karyotypes belonging to populations located at West of Paraná River share only the most common haplotype (DE-14). D-14 is the only haplotype shared by B chromosome carriers on both sides of Paraná River. These results showed that population located at West of the Paraná River exhibited more differences in mt haplotype distribution with respect to karyotype.

Genetic diversity indexes by Region are shown in Table 4. Haplotype diversity for B carriers seems to be lower with respect to standard individuals in the Eastern Region showing lower homogeneity in haplotype distribution in individuals with B chromosomes.

The analysis of molecular variance (AMOVA) in the Eastern Region that accounted for frequencies and divergence between haplotypes revealed that 28% of mtDNA variation was found among karyotypes and the remaining variation could be found among individuals within karyotypes ($\Phi_{ST} = 0.284$; $P = 0.0029$). However, the AMOVAs in the Western Region did not demonstrate significant heterogeneity in mtDNA distribution between karyotypes, all detected variation being attributed to differences among individuals within karyotypes ($\Phi_{ST} < 10^{-4}$; $P = 0.90$).

The Bayesian phylogenetic analysis (Fig. 4) showed two divergent and strongly supported clades. Haplogroup 1 includes haplotypes described only for ST individuals. Haplogrup 2 includes haplotypes belonging to B- carrier individuals of mixed geographical origins. These results indicated that haplotypes are not grouped by their geographical origin; however a well-defined association between karyotype and haplotype is evident.

Discussion

Chromosomal polymorphism in insects has been frequently associated with local adaptation, although the distribution pattern of chromosomal rearrangements may also be caused by historical and environmental factors (Krimbas 1967; Krimbas and Powell 1992; Coluzzi et al. 1979; Hoffmann et al. 2004). In particular, many studies of B chromosome polymorphisms in plants and animals suggested that the presence B chromosomes across populations were related with favourable environments for the species (Jones and Rees 1982; Henriques-Gil et al. 1984).

D. elongatus constitutes an attractive material for the study of karyotype evolution, due to the occurrence of a B chromosome polymorphism widely distributed in Argentina. In the present paper we analysed the pattern of minisatellite loci and COI sequences in polymorphic populations situated at both sides of Paraná River to gain deeper insight about the existence of this chromosome variation in nature.

The DAMD results identified no specific diagnostic band for the different karyotypes. Still it was possible to observe higher genetic diversity on B carriers, estimated by expected heterozygosity and number of bands in B carrier individuals. Moreover the analysis of DAMD dataset showed genetic heterogeneity between karyotypes in the Eastern Region where a higher frequency of B chromosomes has been found (Rosetti and Remis 2012).

There are some previous studies focused to analyse the relationship between B chromosome and the genetic variation in populations. The obtained results varied in different analysed biological models. In rye a negative correlation between the frequency of plants with Bs and the mean heterozygosity at isozymal loci was detected (Benito et al. 1994). In the yellow-necked mouse, *Apodemus flavicollis*, analysis of AFLP loci demonstrated that individuals with and without Bs showed similar levels of genetic variability (Adnadevic et al. 2012).

As in *D. elongatus*, in the Siberian roe deer *Capreolus pygargus* B chromosome frequency is positively correlated with heterozygosity at RAPD loci; additionally, the analysis of SSR markers showed higher genetic variation in the maize land races with 2B with respect to races without Bs (Tokarskaia et al. 2000; Qi-luncc et al. 2007).

Moreover our results of the AMOVA and Bayesian approaches based on DAMD markers suggest an increased genetic differentiation between karyotypes at the East of Paraná River. Likewise, the clustering analysis indicated that the populations in which both B chromosome carriers and standard individuals were separated into different genetic clusters (YAP and GUA), belong to Eastern Region. In previous studies in which individuals were not separately analysed by karyotype (Rosetti and Remis 2012), YAP and GUA were grouped with other populations as a mixed cluster. Sometimes they also group together with populations that are geographically distant, suggesting that when karyotype is considered as a variable, B chromosomes are important factors in determining the population structure of the Eastern Region. The differentiation between B chromosome carriers and standard individuals shown in the multidimensional scaling analysis supports this assumption.

One hypothesis to interpret our results may be that B chromosome carriers could be genetically differentiated from standard individuals. These results would indicate that Bs are associated with minisatellite sequences. Though Bs do not have private bands, the existence of supernumerary chromosomes may lead to greater genetic variability on DAMD loci in B carriers with respect to standard individuals. There are examples showing the presence of repetitive sequences on B chromosomes (Ruiz-Ruano et al. 2015; Ramos et al. 2016; Bugarski-Stanojevic et al. 2016). B chromosomes can evolve by amplification and accumulation of repetitive DNA located originally in some chromosome fragment of the A complement. B chromosomes carrying this repetitive DNA can continue with its independent evolution leading to an increase of molecular variability. Supernumerary chromosomes could eventually exchange genetic material with standard chromosomes driving an even higher genome diversification (Cabral de Melo et al. 2010).

A second possible explanation may be related with the dispersal capability of different karyotypes. In invertebrates, there are many examples in where larger individuals disperse more standing a positive correlation between dispersal and body size (Benton and Bowler 2012). Previous studies showed that B-carriers males in *D. elongatus* have a significantly smaller body size than individuals with standard karyotype (Rosetti et al. 2007). This could enable dissimilar mobility and dispersal capability between karyotypes in males generating different genetic patterns in biparentally inherited DAMD loci (karyotype biased dispersal). This differential dispersion between karyotypes may be more conspicuous in the Eastern Region, due to the greater abundance of B chromosomes detected previously (Rosetti and Remis 2012).

Another probable scenario may be that Bs are better tolerated in individuals with higher variability, and thus the presence of B´s may lead to B carrier individuals having a differential pattern of bands. This pattern is particularly evident in the Eastern Region populations.

It was suggested that B chromosomes are more frequent under particular genetic background because Bs are better tolerated there (Shaw 1984; Parker et al. 1991; Camacho et al. 2000). In plants it was proposed that harmful Bs are better maintained in outcrossed species respect to species under inbreeding (Burt and Trivers 1998). In outcrossed species, which are those with the highest genetic

variability, Bs would be able to affect new lineages, thus evading the loss if they drive (Palestis et al. 2004). The evidence regarding B chromosomes in D. elongatus supports the hypothesis that their persistence in populations is the result of trade-off among opposite selective effects and interactions with their mitotic instability (Rosetti et al. 2008). Under this scenario one is tempted to propose that differential tolerance of B chromosomes in Eastern and Western populations could be due to different effects of antagonistic selection acting on both sides of the Paraná River. However, we cannot rule out any scenario and further studies analysing the presence of minisatellite regions on B chromosome can provide insights about the relationship between karyotype and nuclear genetic variability.

There are scares examples to relate B chromosomes and mt DNA variation in nature. In the grasshopper *Eyprepocnemis plorans* cytogenetic and RFLP analysis across a small area in Southern Spain showed that the presence or absence of B chromosomes was independent of the mt haplotype (Clemente et al. 2001). The authors proposed that the variation in the incidence of B chromosomes and mitochondrial variants may be explained by two different events of replacement.

In *D. elongatus*, mitochondrial AMOVA indicated that variation between karyotypes in the Eastern Region accounts for over 28% of maternally inherited molecular variability suggesting a strong association concerning karyotype variation and molecular differentiation in the mitochondrial genome in the East of Paraná River. By contrast, the Western Region showed no significant difference. The similar distribution of haplotypes in B carriers and standard individuals of Western Region may be related to the fact that populations west of the Paraná River are more ancient and haplotypes are represented similarly in both karyotypes (Rosetti and Remis 2012).

B carrier individuals of the Eastern Region show the D-14; D-13; D-22 and D-26 haplotypes, with the latter three haplotypes being private of the region. We noticed an increased frequency of one of the most common haplotype of the East (DE-26) in B chromosome carriers. This result may be related with the fact that the haplotype distribution in more recently colonized populations of the Eastern Region reflect that the invasion of Bs has occurred in association with certain haplotypes (in particular DE-26), which could be at low frequencies in the Western Region at the moment of colonization; after the event the frequency of DE-26 could have increased in the Eastern Region. An alternative explanation may be related with positive selection processes favouring this haplotype in B carriers of the Eastern Region. The main split in mtDNA Bayesian phylogeny corresponds to the chromosome division between the B carriers and standard karyotype groups. Two haplogroups were underlined in the tree, both showing a robust relationship between haplotype clustering and chromosome constitution. Individuals with Bs from both the Eastern and Western Regions shared haplotypes belonging to Haplogroup 2. These results suggest that the phylogenetic signal is not strongly associated with the geographical distribution but it may have an impact on karyotype distribution patterns. Considering that COI mtDNA sequences as a marker unlinked with the Bs one is tempted to propose that B chromosomes are tolerated in individuals with phylogenetically related mitochondrial DNA haplotypes.

In comparison to mtDNA data, DAMD data showed markedly lower -but still statistically significant- levels of differentiation among karyotypes in the Eastern Region. This difference in population structure for both genetic markers could be due to any of the commonly cited reasons such as stochastic lineage sorting, differences in effective population sizes between nuclear and mitochondrial DNA, differences in mutation rates among genes, and/or sex-biased dispersal.

The present study showed that B chromosome in *D. elongatus* is an important factor in determining the genetic population structure at minisatellite and mitochondrial DNA levels. B chromosome is accompanied with minisatellite and mtDNA sequence variation because of the inherent nature of the B chromosome, or due to karyotype biased dispersal, or in relation to differential tolerance of them in different genetic background. Our findings pointed out that B chromosome may be related with certain genomic variation patterns which may be relevant in the B chromosomes maintenance already proposed depending on the balance of the aforementioned trade-off effects and the possible accumulation mechanisms.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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