GENETIC VARIABILITY IN *Partamona helleri* (Hymenoptera: Apidae) POPULATIONS WITH AND WITHOUT B CHROMOSOMES

VARIABILIDADE GENÉTICA EM POPULAÇÕES DE Partamona helleri *(Hymenoptera: Apidae) COM E SEM CROMOSSOMOS B*

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ABSTRACT: *Partamona helleri* individuals have already been found with up to four B chromosomes. This study assessed whether these chromosomes would have any effect on the *P. helleri* genetic variability. Twenty colonies were selected and divided into three groups: 1) colonies in which no individual possessed B chromosomes; 2) colonies in which some, but not all, individuals possessed B chromosomes, and, 3) colonies in which all the individuals possessed B chromosomes. The DNA from 8 individuals of each colony was analyzed using the PCR-RAPD technique. The heterozygosity values estimated were 0.168, 0.159 and 0.16643 for the three groups, respectively. The molecular analysis of variance showed that 94.16% of the genetic variability was due to differences within the groups, while only 5.84% was distributed among the groups. These data and the graphic dispersion analysis performed suggest that the B chromosomes have no significant effect on *P. helleri* genetic variability.

KEYWORDS: Hymenoptera, *Partamona*, B chromosomes, molecular marker, genetic variability

INTRODUCTION

B chromosomes, also called supernumerary or accessory chromosomes, are extra, non-essential chromosomes that are present in some individuals of some populations of many species. They are usually smaller in size and probably derive from the standard complement of A-chromosomes. These chromosomes follow their own evolutionary path, that is, they do not follow the standard Mendelian segregation (CAMACHO et al., 2000).

B chromosomes have been reported in plants, animals and several fungus species (CAMACHO et al., 2000). These chromosomes have been reported mainly in some taxonomic groups, such as Graminea, Liliacea and Orthoptera, although their high frequency in these groups probably reflects the intensity and the technical facilities of studying them (CAMACHO et al., 2000).

Several studies have already been carried out on these extra elements and their effect on the individuals that carry them. These studies showed that these chromosomes can influence the fitness of organisms that carry them (MIAO et al., 1991), the chiasma frequency (CAMERON and REES, 1967; BELL and; BURT, 1990; CAMACHO et al., 2002, RIERA et al., 2004), the organism fertility (BENITO et al., 1992, ZURITA et al., 1998) or even the individual growth, as in the case of rice (CHENG et al., 2000).

Studies aiming to verify the influence of the presence of these chromosomes on the genetic variability of the individuals that carry them have also been carried out (BENITO et al., 1992; TOKARSKAIA et al., 2000; WÓJCIK et al., 2004).

The hymenopteran species *Partamona helleri* is abundant in southeast Brazil from the north of Santa Catarina state to the south of Bahia state (PEDRO, 1998). In this species, individuals with up to four B chromosomes have been detected (TOSTA et al., 2004). TOSTA et al. (2004) isolated and cloned a RAPD (Random Amplified Polymorphic DNA) marker in *P. helleri* that appeared only in individuals carrying B chromosomes, suggesting the presence of DNA sequences being specific to B chromosomes. But since RAPD markers show repeatability problems, this marker was later transformed to a SCAR (Sequence Characterized Amplified Regions) marker. The SCAR marker based on the partial sequence of the RAPD marker obtained previously by TOSTA et al. (2004) is much more specific and stable and therefore, capable of very efficient identification of carrier individuals of B chromosomes (TOSTA, 2005).

In this study this SCAR marker was used to identify carrier individuals of B chromosomes in different *P. helleri* colonies, in order to verify the existence of effects of these extra supernumerary elements on the genetic variability of the individuals or colonies where they were present.

MATERIAL AND METHODS

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Adult workers from different *P. helleri* colonies present in the Apiário Central at the Universidade Federal de Viçosa, and workers from colonies sampled at different Brazilian locations were analyzed.

DNA was extracted following the protocol recommended by WALDSCHMIDT et al. (1997).

Thirty colonies were analyzed with the pair of SCAR primer (TOSTA, 2005) to verify the presence of B chromosomes. The DNA was amplified using the SCAR primers according to TOSTA (2005). The "Primer A" sequence was CCCGCTACACATATAATTAACG and the "Primer B" sequence was CCCGCTACACCCTAAAATATNC.

Sequentially, 20 of these colonies were choose at random and divided into three groups: 1) colonies in which no individual possessed B chromosomes; 2) colonies in which some, but not all, individuals possessed B chromosomes, and, 3) colonies in which all the individuals possessed B chromosomes. Eight individuals from each one of these colonies were used to estimate their genetic variability, through PCR-RAPD technique (WILLIAMS et al., 1990), using 20 decamer random primers (OPB1, OPB5, OPB6, OPB7, OPB19, OPI2, OPI16, OPI7, OPI18, OPI19, OPK3, OPK5, OPK10, OPN1, OPN2, OPN5, OPN9, OPN12, OPN19, OPR20). The amplification products were separated by electrophoresis in 1.2% agarose gel containing 0.2 ug/ml ethydium bromide, immersed in TBE buffer.

For the statistical analysis of the data, a binary matrix was constructed based on the presence (1) and absence (0) of the amplified DNA fragments. From this matrix the mean number of bands/colony, the number of polymorphic loci/colony and the estimated mean heterozygosity for each one of the three groups of colonies were calculated. This matrix was also used for molecular analysis of variance or AMOVA (EXCOFFIER et al., 1992) and graphic dispersion analysis. All these analyses were carried out using the Genes program (CRUZ, 2005).

RESULTS AND DISCUSSION

In 10 out of the 30 colonies analyzed using the SCAR marker, B chromosomes were not detected in any individual sampled (colonies without B chromosomes or first group). In 12 colonies, individuals with and without B chromosomes were observed (mixed colonies or second group) and in eight colonies B chromosomes were detected in all the individuals analyzed (colonies with B chromosomes or third group) (Table 1).

Table 1. Code, localities, geographic coordinates and number of individuals (N) for the *P. helleri* colonies analyzed with the SCAR primer and with the RAPD primer (*).

Code	Localities	Geographic coordinates	N	Indiv. with B	Indiv. withou B
*902	Rio Vermelho-MG	18º17`LS, 43º00`LW	15	11	4
*903	Rio Vermelho-MG	18º17`LS, 43º00`LW	10	10	-
*RV2	Rio Vermelho-MG	18º17`LS, 43º00`LW	10	10	-
*904	Esmeralda-MG	19º45`LS, 44º18`LW	12	7	5
*BH	Belo Horizonte-MG	19º55`LS, 43º56`LW	10	5	5
BT	Betim-MG	19º58`LS, 44º11`LW	10	10	-
BT2	Betim-MG	19º58`LS, 44º11`LW	9	2	7
*897	Pedra do Anta-MG	20º35`LS, 42º42`LW	10	5	5
BJ1	Teixeiras-MG	20º39ºLS, 42º51`LW	14	-	14
SL	Teixeiras-MG	20º39ºLS, 42º51`LW	12	12	-
592	Porto Firme-MG	20º40`LS, 43º05`LW	21	18	3
*590	Porto Firme-MG	20º40`LS, 43º05`LW	10	10	-
*591	Porto Firme-MG	20º40`LS, 43º05`LW	10	-	10
*896	Piranga-MG	20º41`LS, 43º18`LW	10	-	10
*PK	S. Miguel Anta-MG	20º42`LS, 42º43`LW	10	10	-
SM10	S. Miguel Anta-MG	20º42`LS, 42º43`LW	12	-	12
*603	Viçosa-MG	20º45`LS, 42º52`LW	10	-	10
*588	Viçosa-MG	20º45`LS, 42º52`LW	17	12	5
*UY	Viçosa-MG	20º45`LS, 42º52`LW	10	-	10
*G15	Viçosa-MG	20º45`LS, 42º52`LW	10	10	-

*LT	Viçosa-MG	20º45`LS, 42º52`LW	20	14	6
898	Viçosa-MG	20º45`LS, 42º52`LW	12	8	4
MBI 1	Viçosa-MG	20º45`LS, 42º52`LW	12	9	3
*757	Ribeirão Preto-SP	21º10`LS, 47º48`LW	10	-	10
*858	Ribeirão Preto-SP	21º10`LS, 47º48`LW	10	-	10
*857	Ribeirão Preto-SP	21º10`LS, 47º48`LW	10	5	5
856	Ribeirão Preto-SP	21º10`LS, 47º48`LW	10	-	10
*587	Ribeirão Preto-SP	21º10`LS, 47º48`LW	10	-	10
Sta. 1	Santa Teresa-ES	19º56`LS, 40º36`LW	19	17	2
*Sta. 2	Santa Teresa-ES	19º56`LS, 40º36`LW	10	10	-

In all locations analyzed where found colonies were all or part of the individuals had B chromosomes and colonies in which no individuals had B chromosomes, showing that the presence of B chromosomes is not related to the geographic origin of the colonies.

The RAPD primers used produced 196 DNA fragments, that is, 10 bands/primer on average. In the colonies where all the individuals had B chromosomes, 52.5% of the fragments observed showed segregation, an identical result to that observed in the colonies without B chromosomes, while 50% of the fragments segregated in the group of the mixed colonies. None of the primers used produced molecular markers (private bands present in only one of the groups), what might help in their genetic characterization.

Great similarity was detected in the pattern of the RAPD bands, both among and within the groups, what generated very close expected mean heterozygosity values for the three groups (H_e = 0.168, 0.159 and 0.166, for groups 1, 2 and 3, respectively). In spite of criticism regarding the calculation of heterozygosity, when using dominant markers and assuming the Hardy-Weinberg equilibrium, this parameter was calculated to give an idea of the segregation of the loci in the three groups and, consequently of the influence of the chromosomes on the genome of the individuals.

The analysis of molecular variance (ANOVA) showed that 94.16% of the genetic variability was due to within group differences, while only 5.84% was distributed among the groups, which confirmed the high homogeneous RAPD patterns among the groups and the absence of specific molecular markers for each one of the three groups established. The high genetic similarity among the bees in the three groups analyzed was also confirmed by the formation of a single group in the graphic dispersion analyses (Figure 1).

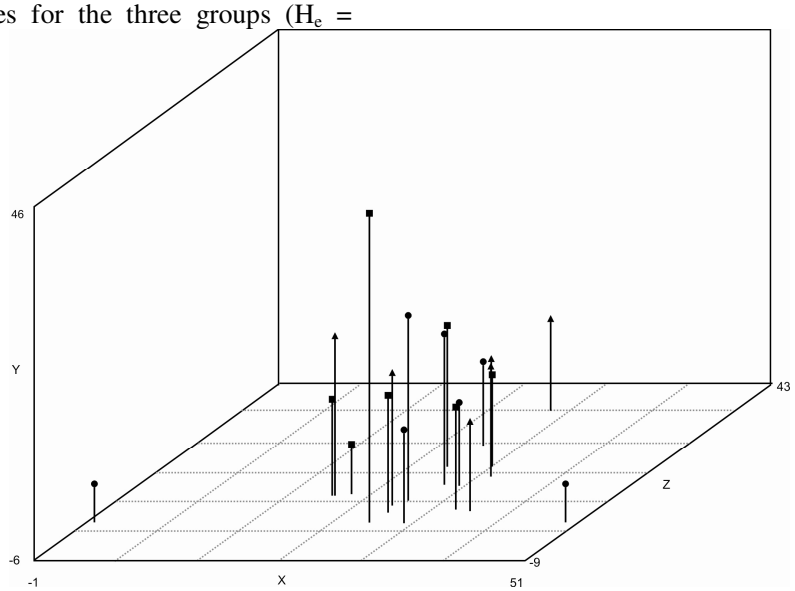

Figure 1. Graphic dispersion analysis showing the genetic differences between the *P. helleri* colonies analyzed. Spheres: colonies without B chromosomes; squares: mixed colonies and triangles: colonies with B chromosomes.

The results obtained therefore suggested that the B chromosomes do not have any significant effect on *Partamona helleri* genetic variability, estimated by the PCR-RAPD technique, as was reported for enzymatic analyses of mice with B chromosome (WÓJCIK et al., 2004). However, BENITO et al. (1992) reported negative correlation between the frequency of plants with B chromosomes and mean heterozygosity when they studied four *Secale cereale* Korean populations containing B chromosomes, using the isoenzyme technique. TOKARSKAIA et al. (2000) detected a positive relationship between B chromosomes presence and increase in variability when studying the intra and inter specific variation among two species of deer of the *Capreolus* genus using PCR-RAPD. These data show that the effect of B chromosomes on individuals can vary from species to species or from population to population.

These authors, however, does not explain how the B chromosomes could specifically affect the species genetic variability, but according to WÓJCIK et al. (2004), the adaptive effects of B chromosomes could result from their influence on the overall genetic variation in populations.

CAMACHO et al. (2000) stated that B chromosomes can have several effects in the fitness of the individuals carrying them. One of the most reported B chromosome effects is on the quiasma frequency and, therefore, recombination. B chromosomes are frequently associated with increases in chiasma frequency, although cases of absence of effects, and even chiasma frequency decrease associated with B presence have also been reported (JONES and REES, 1982). Several explanations for the effects of B chromosomes on chiasma and crossing-over have been proposed. One widely discussed theory proposed that an increase in chiasma frequency increases genetic variability and thus enables population to evolve more rapidly (JOHN and HEWITT, 1965; HEWITT and JHON, 1967), whereas a decrease conserves variability and helps to maintain adaptation (FONTANA and VICKERY, 1973). BELL and BURT (1990) criticized this idea. Instead, they suggested that B chromosomes act as parasites that evoke a response in the host organism. One response of the host is an increase in crossing-over, which may serve to produce a great variety of offspring and a better chance of developing individuals that block accumulation of the parasitic Bs.

Thus, further studies are necessary to improve our understanding about the origin and evolution of B chromosomes in *P. helleri* in order to analyse their contribution to the genetic variability of this species.

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RESUMO: Em *Partamona helleri* já foram encontrados indivíduos com até quatro cromossomos B. Neste trabalho, avaliou-se se estes cromossomos teriam algum efeito sobre a variabilidade genética de *P. helleri*. Vinte colônias foram selecionadas e divididas em três grupos: 1) colônias nas quais nenhum indivíduo possuía cromossomos B; 2) colônias nas quais alguns indivíduos possuíam cromossomos B e outros não e, 3) colônias nas quais todos os indivíduos possuíam cromossomos B. O DNA de 8 indivíduos de cada colônia foi analisado através da técnica de PCR-RAPD. Os valores da heterozigosidade estimada foram 0,168, 0,159 e 0,166 para os três grupos, respectivamente. A análise molecular de variância demonstrou que 94,16 % da variabilidade genética foi devida a diferenças dentro dos grupos, enquanto apenas 5,84% estava distribuída entre os mesmos. Estes dados e a análise de dispersão gráfica realizada sugerem que os cromossomos B não têm nenhum efeito significativo sobre a variabilidade genética de *P. helleri.*

PALAVRAS-CHAVE: Hymenoptera. *Partamona*. Cromossomos B. Marcador molecular. Variabilidade genética

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