

SYSTEMATICS, MORPHOLOGY AND PHISIOLOGY

RAPD Variation within and among Species of *Melittobia* Westwood
(Hymenoptera: Eulophidae)

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Neotropical Entomology 35(2):201-205 (2006)Variação de Marcadores RAPD dentro e entre Espécies do Gênero *Melittobia* Westwood
(Hymenoptera: Eulophidae)

RESUMO - O gênero *Melittobia* Westwood compreende várias espécies de microparasitóides, das quais apenas duas foram registradas no Brasil até agora: *M. australica* Girault e *M. hawaiiensis* Perkins. Entretanto, essas espécies são de difícil identificação através de métodos taxonômicos tradicionais. No presente trabalho, marcadores moleculares amplificados ao acaso (PCR-RAPD) foram utilizados com o objetivo de se discriminar as duas espécies e, ao mesmo tempo, analisar a variabilidade genética em populações de *M. australica*. A maioria dos fragmentos gerados foi espécie-específicos estando presente em todos os indivíduos de uma espécie e ausente nos indivíduos da outra espécie, demonstrando a adequação dessa técnica na distinção das espécies de *Melittobia* estudadas. A técnica de PCR-RAPD também demonstrou que os indivíduos das diferentes populações estudadas são muito semelhantes entre si, o que pode ser atribuído ao efeito fundador e/ou a grande capacidade de dispersão dessas populações. As distâncias genéticas dentro ($D = 1,19-3,54\%$) e entre as populações ($D = 1,93-5,28\%$) de *M. australica* apresentaram valores muito baixos, refletindo a reduzida variação genética existente nas populações dessa espécie.

PALAVRAS-CHAVE: *Melittobia australica*, *M. hawaiiensis*, marcador molecular, variabilidade genética, microparasitóide

ABSTRACT - The genus *Melittobia* Westwood comprises several species of microparasitoids and only two of them are know to occur in Brazil up to now: *M. australica* Girault and *M. hawaiiensis* Perkins. Nevertheless, the differentiation between these two species using traditional taxonomy is very difficult. In the present study, we used random amplified polymorphic DNA chain reaction (RAPD-PCR) to test for its ability to discriminate between these two species and to examine the genetic variation among the studied populations of *M. australica*. Most of the generated fragments were species-specific, occurring in all individuals of one species and absent in the individuals of the other species demonstrating the appropriateness of such technique to distinguish between both of the *Melittobia* species occurring in Brazil. RAPD-PCR also demonstrated low variability among different populations of *M. australica*, which may be due to a founder effect and/or high dispersion capacity of these populations. Genetic distances within ($D = 1.19-3.54\%$) and among populations ($D = 1.93-5.28\%$) presented very low values, reflecting the reduced genetic variation that exists among populations of *M. australica*.

KEY WORDS: *Melittobia australica*, *M. hawaiiensis*, molecular marker, genetic variability, microparasitoid

Melittobia Westwood are microhymenopteran wasps (1-3 mm length) that parasitize larvae and pupae of several species of bees and wasps, with one species being recorded as pest due its parasitism of commercially reared pollinators (Dahms 1984ab, Whitfield & Cameron 1993, Wael *et al.* 1995).

Melittobia are gregarious ectoparasites and the females

lay eggs directly on the host (Matthews 1997), in batches isolated from one another. This ensures that males and females from the same mother emerge in spatial and temporal proximity to one another (Dahms 1984b). Males are generally brownish in colour, have no eye pigments, have the scape expanded, are brachypterous and emerge before

females. They are confined to the immediate area of their emergence. They locate each other and fight often to death for the chance to court their siblings as they emerge (Dahms 1984b). Females are smaller than males, dark brown, have red eyes, normal wings and antennae without large scape. After mating, females disperse to colonize a new host, and their brothers die, never having left the nest (Balfour-Browne 1922, Cooperband *et al.* 2003). Therefore, due to endogamy, low genetic variability can be expected among individuals of each population.

This low genetic variability may be accentuated by the fact that females become non-receptive after insemination (Askew 1968). But this is controversial since others considered that *M. australica* and *M. acasta* females can and do mate a second time, with a son, when their sperm supply is depleted (Balfour-Browne 1922, Dahms 1984b). However, relatively little is known about the extent and distribution of genetic diversity in natural populations of *Melittobia*.

In Brazil only two *Melittobia* species were recorded up to now: *M. australica* Girault and *M. hawaiiensis* Perkins. Females from these species are indistinguishable and males can be externally differentiated uniquely by the number of setae on the antennae scape (Dahms 1984a). Thereby, the distinction between *M. australica* and *M. hawaiiensis* is very complex, not only because this character is difficult to analyse, but also because males are found in low number. In *M. australica*, for example, the proportion of males is only 1-4% (Dahms 1984b). In this sense, it is important to investigate other alternative methods to discriminate these species.

The use of molecular markers, such as RAPD has been quite helpful in distinguishing species and identifying genetic variations between and within Hymenoptera populations. RAPD markers can be efficient for the study of genetic variability because they make possible the random amplification of many genome regions that can be compared simultaneously. Furthermore, RAPD technique does not require large amounts of DNA nor any previous knowledge about the genome sequences of the species under investigation (Kazmer *et al.* 1995, Lu & Rank 1996, Lou *et al.* 1998).

In this paper we applied PCR-RAPD technique to distinguish the two *Melittobia* species recorded in Brazil and also to describe the RAPD variability among and within different populations of *M. australica*.

Materials and Methods

Samples from *M. australica* and from *M. hawaiiensis* were collected in Viçosa (Minas Gerais State) and in Senador Guimard (Acre State), respectively. Specimens were identified by Dr. John LaSalle from England. They were originally collected in the field as adults parasitizing *Trypoxylon* Latreille (Hymenoptera: Sphecidae) larvae and were maintained in the laboratory. Twelve adult females of each species were used in the analysis to distinguish them. Adult males from both species were used in parallel tests to confirm species' amplification patterns.

The analyses of intraspecific genetic variability were performed in four populations (IEF, Acamari, Apiário and Vila Secundina) of *M. australica* collected in Viçosa and

one population collected in São José dos Campos (Sao Paulo State). Each population was obtained from a single *Trypoxylon* hive. Twelve adult males from each population were analyzed. Given the small proportion of males in each population, progeny by more than one *M. australica* female was used for analyses. For each population collected in Viçosa, ten different females were isolated from the original population and put in a clean bottle with a host larva to obtain a new generation of females and the studied males. Six female sisters contributed to generate males in the IEF population, while eight female sisters contributed to generate males in the Acamari, Apiário and Vila Secundina populations. Males from São José dos Campos, however, were collected directly from the original population, and the number of female sisters that originated them is unknown.

DNA extraction and amplification. DNA was extracted following the protocol described by Waldschmidt *et al.* (1997) and amplified by PCR-RAPD as described by Williams *et al.* (1990), with some modifications. Each PCR reaction was set up in a total volume of 25 µl with 1X reaction buffer, 0.1 mM dNTPs, 0.4 µM 10-base primer (Operon Technologies, Alameda, CA, USA), 0.5 U Taq polymerase (Phonutria, Belo Horizonte, MG, Brazil) and 3 µl DNA template (with non quantifiable concentration). Tubes with reaction mixes were placed into a PTC-100 thermocycler (MJ Research) programmed for 45 cycles. Each cycle consisted of 92°C for 30s; 35°C for 1 min; 3 min 42 s ramp to 72°C; 72°C for 2 min. After the 45th cycle a final extension step at 72°C for 7 min was performed.

Ten RAPD primers (OPB-05, OPC-06, OPF-16, OPH-09, OPM-14, OPP-10, OPR-02, OPS-05, OPT-07 and OPU-12) were used to discriminate between the two species, while 17 RAPD primers (OPB-05, OPF-15, OPF-16, OPG-04, OPG-15, OPH-09, OPI-17, OPJ-10, OPM-07, OPP-10, OPQ-10, OPS-04, OPT-11, OPU-12, OPU-13, OPX-04 and OPZ-14) were used to analyse the genetic variability among different populations (Table 1). In order to avoid possible contaminants influence in all analyses, a negative control (using sterile water instead of DNA template) was used for each amplification with a single primer. Amplification products were checked on 1.2% agarose gels in TBE buffer (90 mM Tris-borate pH 8.0, 10 mM EDTA) and stained with ethidium bromide (0.2 µg/ml). Bands were photographed under UV light.

Discrimination between species. Amplification products (24 individuals) obtained for the two species, with the same primer, were analysed on a single gel. Only bands which were bright, reproducible and absent in the negative control reaction were scored. PCR amplification products were scored as present (1) or absent (0) and data were used to generate a pairwise genetic distance matrix with values calculated using Nei-Li coefficient (Cruz 2001). Discrimination between species was visualised by this matrix' projection in the bidimensional plan using the computer program GENES (Cruz 2001).

Analysis of genetic variability. Amplified DNA fragments obtained for the 60 males were used to generate a binary

Table 1. RAPD primers used for amplifications of the *Melittobia australica* and *M. hawaiiensis* genome.

Primer	Sequence 5' - 3'	Number of bands	
		<i>M. australica</i>	<i>M. hawaiiensis</i>
OPB-05 ^{a, b}	TGCGCCCTTC	8	5
OPC-06 ^a	GAACGGACTC	3	5
OPF-15 ^b	CCAGTACTCC	4	-
OPF-16 ^{a, b}	GGAGTACTGG	8	3
OPG-04 ^b	AGCGTGTCTG	8	-
OPG-15 ^b	ACTGGGACTC	7	-
OPH-09 ^{a, b}	TGTAGCTGGG	8	4
OPI-17 ^b	GGTGGTGATG	6	-
OPJ-10 ^b	AAGCCCAGAG	14	-
OPM-07 ^b	CCGTGACTCA	10	-
OPM-14 ^a	AGGGTCGTTC	9	8
OPP-10 ^{a, b}	TCCCGCCTAC	4	2
OPQ-10 ^b	TGTGCCCGAA	5	-
OPR-02 ^a	CACAGCTGCC	9	10
OPS-04 ^b	CACCCCCTTG	3	-
OPS-05 ^a	TTTGGGGCCT	5	3
OPT-07 ^a	GGCAGGCTGT	6	7
OPT-11 ^b	TTCCCCGCGA	6	-
OPU-12 ^{a, b}	AGACCCAGAG	8	5
OPU-13 ^b	GGCTGGTTCC	7	-
OPX-04 ^b	CCGCTACCGA	9	-
OPZ-14 ^b	TCGGAGGTTC	4	-

^aPrimers used for discrimination between the two species.
^bPrimers used to analyse the genetic variability among populations of *M. australica*.

matrix where data were coded as (1) for band presence and (0) for absence. The data matrix was used for calculation of the Jaccard's similarity index (S_{ij}), within each family and among pairs of families, in which the genetic similarity between the *i*th and *j*th individuals is given by:

$$S_{ij} = a/(a + b + c)$$

where

- a = number of bands present in the two samples (1,1)
- b = number of bands present in the first sample and absent in the second (1,0)
- c = number of bands absent in the first sample and present in the second (0,1).

The genetic similarity (S_{ij}) was then converted to genetic distances (D_{ij}) using the arithmetic complement of similarity given by the expression: $D_{ij} = 1 - S_{ij}$.

Results and Discussion

RAPD-PCR analysis proved to be very useful to differentiate between *M. australica* and *M. hawaiiensis*. Most bands (113 bands; 94.2%) were unique to each species, with only seven (5.8%) being common to both of them (Fig. 1). This great interspecific variability in RAPD patterns revealed species-specific profiles for the two *Melittobia* species analysed, making their DNA-based distinction possible.

M. australica presented a higher mean number of bands per primer (6.8) than *M. hawaiiensis* (5.2), demonstrating there are more annealing sites for the RAPD primers tested in the genome of the former. Besides that, polymorphisms between individuals within a species were rare, characterizing each species very properly.

The 2D projection of the genetic distances among individuals (Fig. 2) showed that all *M. australica* individuals

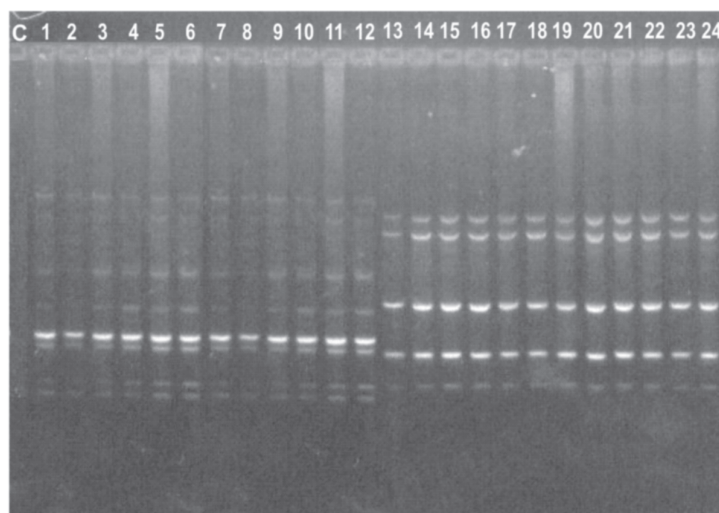


Fig. 1. Representative RAPD amplification patterns of genomic DNA of *M. australica* (lanes 1-12) and *M. hawaiiensis* (lanes 13-24), using the primer OPC-06. "C" is the negative control reaction.

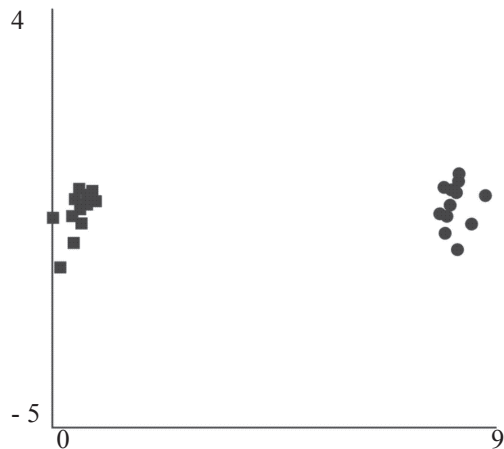


Fig. 2. 2D projection based on Nei-Li coefficient of RAPD band frequency within populations of *M. australica* (●) and *M. hawaiiensis* (■).

were closely grouped and distinct from *M. hawaiiensis* individuals. The close association between individuals of the same species reflects well its endogamic origin and the fact that the majority of genetic variation is between rather than within species, reinforcing that both species could be clearly discriminated.

In the analyses of the genetic variability of the five *M. australica* populations, bands that were not clearly identifiable were excluded from the final dataset. Therefore, the resulting binary matrix was composed by 87 bands, of which, only 11 were polymorphic in different populations. The mean number of polymorphic bands/primer was 1.47, a value similar to that obtained in the analysis of the progeny and populations of other haplodiploid insects (Hunt & Page 1992, Waldschmidt *et al.* 2002).

Analysis of the RAPD markers profiles obtained for *M. australica* pointed to a high band homogeneity among males of each population (Fig. 3) confirming a high genetic

similarity within populations (Table 2), as expected for an endogamic species.

The genetic distance between pairs of populations (Table 2) also assumed very low values. The genetic distance between populations from Acamari and Vila Secundina, for example, was lower than all other distances between and within most populations, indicating that individuals from these two populations are as similar to each other as individuals within each population. This low variability among populations could be explained by founder effects and/or a high dispersion capacity of females facilitated by man and wind.

M. australica was originally described from Australia, but it has also been found in Japan, New Zealand, Trinidad, South Africa, USA, Venezuela, Bahamas, Costa Rica, Cuba, Hispaniola, Puerto Rico and Trinidad (Dahms 1984a, La Salle 1993, González & Terán 1996, González *et al.* 2004). Human global mobility has provided *Melittobia* with a new means of dispersal in a way that a small number of *M. australica* could have been accidentally introduced in Brazil in ships or in packing crates and rapidly expanded throughout the country helped by air currents.

According to Dahms (1984b), several features of *Melittobia*'s biology could have aided in this process. As *Melittobia* are highly polyphagous, the presence of mud nesting Hymenoptera and cockroach oothecae in ships would have served as hosts for *Melittobia* females. Females are also able to delay feeding and oviposition for several weeks until a host is at a suitable stage for oviposition or until a suitable host is located. Anyway, modern, rapid transport reduces the risk of *Melittobia* females dying without finding a suitable host. Most interestingly, *Melittobia* females have the capacity to be very efficient founder organisms. It is theoretically possible for an unseminated female to begin a new population by laying a few unfertilized eggs. These develop into males that will mate with her.

Therefore, considering that a small number of females could have been introduced in Brazil, that air currents could

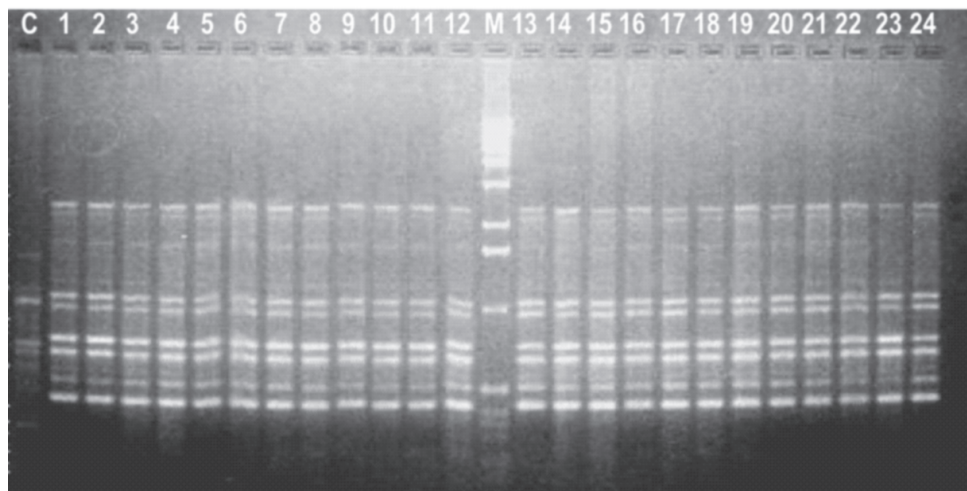


Fig. 3. Representative RAPD amplification patterns of genomic DNA of two *M. australica* populations (Acamari and Vila Secundina, respectively), using the primer OPH-09. "C" is the negative control reaction and "M" is the marker.

Table 2. Genetic distances (%) between and within populations of *M. australica*.

Families	1	2	3	4	5
1. Apiário, Viçosa	2.63				
2. IEF, Viçosa	3.71	3.54			
3. Acamari, Viçosa	4.43	4.22	1.19		
4. Vila Secundina, Viçosa	5.02	5.06	1.93	2.51	
5. São José dos Campos – São Paulo	5.28	5.04	3.95	4.21	3.25

have provided the introduced females with added means of dispersal and that *Melittobia* uniseminated females have the capacity to begin a new population by laying a few unfertilized eggs, distantly geographic populations could be genetically similar.

Our data do support the hypothesis that genetic distances among populations are not directly related to geographic distances, because even when we compare more distant populations (Viçosa/MG and São José dos Campos/SP), the genetic divergence values were low. In some cases, the genetic distance between two populations from Viçosa was greater than distance between two populations from São José dos Campos and a population from Viçosa (Table 2).

Nevertheless, to a better understanding of the genetic variability of *M. australica* it would be necessary to study a greater number of populations. This could improve the genetic knowledge about this species as well as its evolutive and ecological interactions within the ecosystems as a whole.

Acknowledgments

The authors thank Dr. Cosme Damião Cruz from the Universidade Federal de Viçosa for statistical assistance with GENES Program.

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Received 28/III/05. Accepted 12/IX/05.