ACAROLOGY

Genetic Similarity among *Agistemus pallinii* Matioli *et al* (Acari: Stigmaeidae) Found in Citrus Orchards in Viçosa, Minas Gerais State, Brazil

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Similaridade Genética entre *Agistemus pallinii* Matioli *et al* (Acari: Stigmaeidae) Encontrados em Pomares de Citros em Viçosa, MG

RESUMO - Stigmaeidae são importantes predadores de ácaros e insetos pragas em várias culturas no Brasil. Essa família é considerada a segunda mais importante de ácaros predadores em citros no Brasil. Entretanto, o status taxonômico desses ácaros, principalmente do gênero *Agistemus*, é difícil de ser analisado com base em caracteres morfológicos. Assim, este trabalho descreve o uso de marcadores polimórficos amplificados ao acaso (PCR-RAPD) para investigar a similaridade genética de uma população de *Agistemus pallinii* Matioli *et al* encontrada em 2004 em pomar de citros em Viçosa, MG, predando *Panonychus citri* (McGregor). As amplificações foram realizadas com 12 iniciadores randômicos (OPAA8, OPAA19, OPAB1, OPAB5, OPAB18, OPAC9, OPAC17, OPAC19, OPAD10, OPAE9, OPAE12 e OPAE17), os quais geraram 119 bandas, sendo 53,8% polimórficas. Os coeficientes de similaridade genética entre os indivíduos variaram de 0,68 a 0,99, indicando alta similaridade entre eles. A análise de projeção gráfica 3D mostrou que a maioria dos indivíduos analisados formou um único grupo, confirmando a alta similaridade genética entre os mesmos. Assim, mesmo considerando o pequeno tamanho de *A. pallinii* (± 360 μm), a técnica de PCR-RAPD pode ser utilizada para a sua identificação, em suporte às análises morfológicas ou em comparações de populações coletadas em diferentes regiões geográficas. O presente trabalho representa o primeiro estudo molecular realizado com ácaros da família Stigmaeidae.

PALAVRAS-CHAVE: Ácaro predador, variabilidade genética, marcador molecular

ABSTRACT - Stigmaeidae are very important predators of mite and insect pests on several crops in Brazil. It is considered the second most important family of predatory mites in citrus orchards in Brazil. However, their identification, especially that of the members of the genus *Agistemus*, is rather difficult based only on morphology. Hence, this study describes the use of random amplified polymorphic DNA (RAPD-PCR) markers to determine the genetic similarity of an *Agistemus pallinii* Matioli *et al* population found in 2004 in a citrus orchard in Viçosa, Minas Gerais State, Brazil, preying on *Panonychus citri* (McGregor). Amplifications were performed with 12 random primers (OPAA8, OPAA19, OPAB1, OPAB5, OPAB18, OPAC9, OPAC17, OPAC19, OPAD10, OPAE9, OPAE12 and OPAE17), which generated 119 bands, with 53.8% polymorphism. The coefficients of genetic similarity among the individuals ranged from 0.68 to 0.99, indicating a high genetic similarity among them. The 3D projection analysis clustered the majority of individuals confirming their high similarity. Though individuals of *A. pallinii* are minute (± 360 μm long), the PCR-RAPD technique can still be used for their identification, complementing morphological analyses or for comparison of populations collected in different geographic regions. This is the first molecular study carried out with stigmaeid mites.

KEY WORDS: Predatory mite, genetic variability, molecular marker

Stigmaeidae mites are important predators on several crops in Brazil, such as citrus, coffee, apple, rubber tree and guava, controlling phytophagous and also fungivorous mites (Matioli *et al* 1998, Ferla & Moraes 2003).

Identification of these mites is primarily based on morphologic and taxonomic characters and by crossbreeding species. However, these protocols are sometimes insufficient, especially for *Agistemus,* making necessary the use of other methods to identify them.

The application of molecular markers to studies on mites recently yielded new insights into their population structures and taxonomic relationships. Molecular markers, such as those generated by RAPD-PCR proved very useful in distinguishing species and in identifying genetic variations within and among populations (Perrot-Minnot & Navajas 1995, De Guzman *et al* 1997, Edwards *et al* 1997, Hance *et al* 1998, Yli-Mattila *et al* 2000). Edwards *et al* (1998), for example, used this method to compare three similar species of phytoseiid mites: *Typhlodromalus limonicus* Garman & McGregor, *T. manihoti* Moraes and *T. tenuiscutus* McMurtry & Moraes, concluding that this technique could be used to distinguish other cryptic species, in addition to interbreeding experiments.

Though the Stigmaeidae is considered the second most important predatory mite family in citrus orchards, this method has not yet been applied to their identification or to the comparison of populations collected in different geographic regions. Therefore, in here we report the use of random amplified polymorphic DNA (PCR-RAPD) markers to investigate the genetic similarity of an *Agistemus pallinii* Matioli *et al* population found in citrus orchards in Viçosa, Minas Gerais State, Brazil, preying on *Panonychus citri* McGregor (Acari: Tetranychidae).

Material and Methods

Mites were sampled in a citrus orchard in Viçosa-MG, Brazil, in 2004, and reared under controlled conditions (25°C, 12:12h L:D, and 75% RH). *Brevipalpus phoenicis* (Geijskes) and *P. citri*, supplemented with *Typha* sp. pollen, were offered as a rearing diet (Matioli *et al* 2007), using citrus leaves as a substrate.

DNA extraction of 33 *A. pallinii* females followed Groot *et al* (2005). Individual specimens were placed in a centrifuge tube (0.5 ml) kept in ice, and females were homogeneized in 3 µl of proteinase K (20 mg/ml). Subsequently, 50 µl of a 5% Chelex100® solution was added, samples were briefly vortexed, incubated at 37°C for 1h and quickly vortexed again after 0.5h. Subsequently, the samples were incubated at 96°C for 8 min and briefly agitated. The extracted DNA was used immediately or stored at -20°C for two or three weeks for later use.

DNA amplification. The DNA of individual mites was 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 (Phoneutria, Belo Horizonte, MG, Brazil) and 3 µl DNA template (unknown concentration), and runin a PTC-100 thermocycler (MJ Research) programmed at 92°C for 30 s, 35°C for 1 min, 3 min and 42 s. to increase to 72°C; 72°C for 2 min (45 cycles); 72°C for 7 min (1x).

Amplification products were separated in 1.2% agarose gels containing 0.2 µg/ml of ethidium bromide in TBE buffer (Tris-borate 90 Mm, pH 8.0, EDTA 10mM). Bands were visualized under UV light and the images were analyzed using the AlphaDigidoc 1201 system (AlphaDigidoc™). A negative control without genomic DNA was used in each amplification to check for possible contaminants.

Initially, DNA of six specimens of *A. pallinii* was used in amplification tests to verify the amplification patterns of the RAPD primers. In these amplifications 56 primers were tested and 34 produced amplifications patterns that could be used in subsequent analyses. However, due to the small amounts of DNA available, the subsequent amplifications were carried out only with 12 primers: OPAA8, OPAA19, OPAB1, OPAB5, OPAB18, OPAC9, OPAC17, OPAC19, OPAD10, OPAE9, OPAE12 and OPAE17 (Table 1).

The amplicons produced were scored as present (1) or absent (0), and the constructed data matrix was used for the calculation of the Jaccard´s similarity index (S_{ij}) among the pairs of individuals. The genetic similarity (S_{ij}) was then converted to genetic distances (D_{ij}) using the arithmetic complement of similarity given by the expression: $D_{ij} = 1-S_{ij}$. This matrix was submitted to a 3D graphic dispersion analysis. The projection efficiency of the distances in the plane was estimated according to the correlation between the original distances and those showed in the graph. All analyses were carried out using the Genes program (Cruz 2005).

Results and Discussion

The method used in the present work for the extraction of DNA of individual mites was appropriate for the RAPD-

Table 1 RAPD primers used in DNA genomic amplifications of *Agistemus pallinii*.

Primer	Sequence 5' – 3'	Nº bands	Nº polymorphic bands
OPAA08	TCCGCAGTAG	4	4
OPAA19	TGAGGCGTGT	8	1
OPAB01	CCGTCGGTAG	15	13
OPAB05	CCCGAAGCGA	14	7
OPAB18	CTGGCGTGTC	11	6
OPAC09	AGAGCGTACC	12	5
OPAC17	CCTGGAGCTT	5	0
OPAC19	AGTCCGCCTG	10	4
OPAD10	AAGAGGCCAG	9	6
OPAE09	TGCCACGAGG	10	6
OPAE12	CCGAGCAATC	9	4
OPAE17	GGCAGGTTCA	12	8

PCR amplifications, despite the small size of the mites and, consequently, the small amount of DNA. Our data corroborated that of Yli-Mattila *et al* (2000), showing the suitability of Chelex 100® in extracting DNA from *Euseius finlandicus* Oudmans (Acari: Phytoseiidae). The main limitation was the small number of possible amplifications that can be carried out with the same sample.

Amplifications carried out with the 12 selected primers produced 119 bands, with a range of 4 to 15 bands/primer (Table 1). The mean number of bands produced per primer (9.9) was close to that found for three species of *Typhlodromalus* (8.1) (Edwards *et al* 1997) and different strains of *Tetranychus* sp (8.7) (Hance *et al* 1998).

In general, the PCR-RAPD patterns generated were clear and similar in number among individuals, as the one obtained with the primer OPAE12 (Fig 1), allowing the efficient characterization of the population of *A. pallinii* analyzed in the present study. However, as the absence of one determined band in only one specimen was considered as a polymorphism, 53.8% of the bands generated (64 bands) were polymorphic in the analyzed population.

The RAPD technique had already been used for population analyses and identification of different mite species and/or strains. Hernandez *et al* (1998) obtained RAPD patterns capable of differentiating strains of *Boophilus microplus* (Canestrini) susceptible from resistant ones. Edwards *et al* (1998) examined variable RAPD markers in three *Typhlodromalus* species and found several bands that could be used to distinguish phytoseiid species, while Yli-Mattila *et al* (2000) tested 20 primers in order to determine specific RAPD products to distinguish between strains and mite species.

The mean coefficient of similarity obtained for paired comparisons among the 33 of *A. pallinii* was 0.85 (SD = 0.066), with values ranging from 0.68 to 0.99. The highest similarity (0.99) was observed between 8 and 27, while the lowest (0.68) similarity was between specimens 23 and each of 29 to 32.

This high similarity could be confirmed in the dispersion graphic analysis (Fig 2) where the distance between points

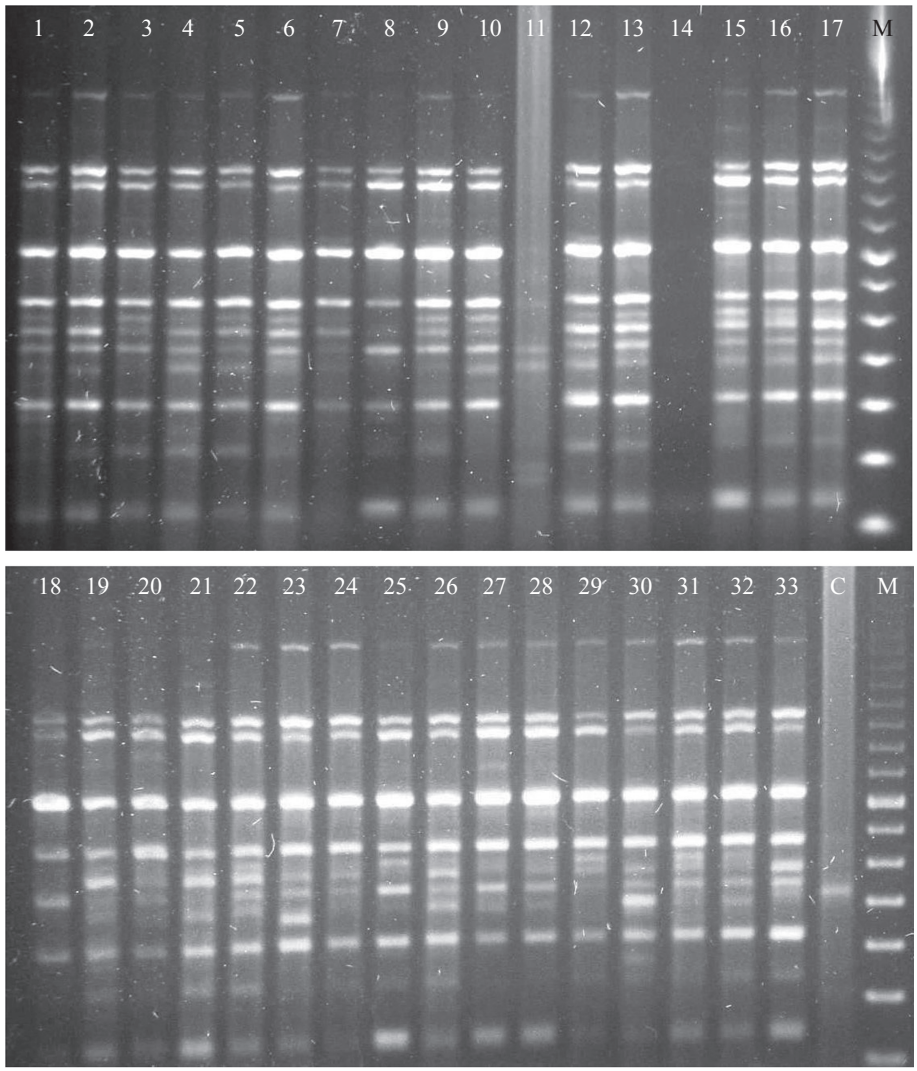

Fig 1 Representative RAPD amplification pattern of genomic DNA of *Agistemus pallinii* using the primer OPAE12. (M: molecular weight marker; C: negative control)

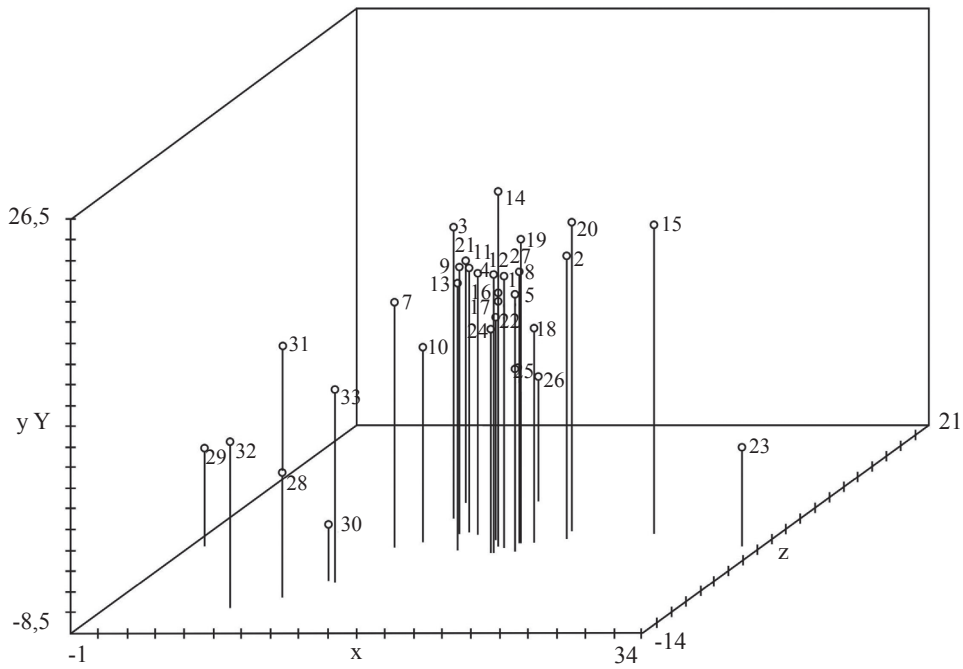

Fig 2 Projection in three-dimensional space of genetic distance on 33 *Agistemus pallinii* specimens collected in Viçosa, MG, Brazil.

is proportional to the dissimilarity between genotypes. Thus, the majority of the analyzed specimens grouped together confirming the high similarity between them, while the more dissimilar individuals (28, 29, 30, 31, 32, 33 and 23) were more distant graphically. The correlation value (81.5%) obtained from this analysis further showed a good adjustment between the genetic distances and the distances graphically represented.

Strong characters to distinguish among species of *Agistemus* are limited, most consisting of ratios, easily influenced by the mounting of the mites. Thus, the RAPD-PCR technique can be a useful tool for species identification. Another advantage is that the species identity can be determined quicker. However, this technique must not replace the taxonomy based on morphology. Hance *et al* (1998), for example, used this technique to compare strains of *Tetranychus* sp. to complement fecundity and biometry studies. Additionally, the loss of information due to the dominance of the technique can be minimized by increasing the number of loci analyzed (Miller 2000). Through cloning and sequencing of RAPD products it is also possible to develop more specific primers (SCARs). Amplification with these primers could result in a more precise identification of species as demonstrated with the identification of different strains of *E. finlandicus* (Yli-Mattila *et al* 2000).

This is the first molecular study carried out with predatory mites of the genus *Agistemus* (Stigmaeidae), whose species is difficult to determine based on morphologic characters only. The RAPD technique revealed to be a promising tool for the identification of species as well as in intra and inter specific diversity studies and serving as a basis for future studies related to other *Agistemus* species.

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