Analysis of the genetic diversity in *Metopolophium dirhodum* (Walker) (Hemiptera, Aphididae) by RAPD markers

Marcelo Lopes-da-Silva[1] & Luiz Gonzaga Esteves Vieira[2]

[1]Curso de Agronomia e Ciências Biológicas, Universidade de Cruz Alta-UNICRUZ, 98025-810 Cruz Alta-RS, Brasil. mlopes@unicruz.edu.br
[2]Laboratório de Biotecnologia Vegetal, Instituto Agronômico do Paraná, Londrina-PR, 86001-970 Londrina-PR, Brasil. lvieira@iapar.br

ABSTRACT. Analysis of the genetic diversity in *Metopolophium dirhodum* (Walker) (Hemiptera, Aphididae). The emergence of host-races within aphids may constitute an obstacle to pest management by means of plant resistance. There are examples of host-races within cereals aphids, but their occurrence in Rose Grain Aphid, *Metopolophium dirhodum* (Walker, 1849), has not been reported yet. In this work, RAPD markers were used to assess effects of the hosts and geographic distance on the genetic diversity of *M. dirhodum* lineages. Twenty-three clones were collected on oats and wheat in twelve localitites of southern Brazil. From twenty-seven primers tested, only four primers showed polymorphisms. Fourteen different genotypes were revealed by cluster analysis. Five genotypes were collected only on wheat; seven only on oats and two were collected in both hosts. Genetic and geographical distances among all clonal lineages were not correlated. Analysis of molecular variance showed that some molecular markers are not randomly distributed among clonal lineages collected on oats and on wheat. These results suggest the existence of host-races within *M. dirhodum*, which should be further investigated using a combination of ecological and genetic data.

KEYWORDS. Rose-Grain Aphid; genetic diversity; host-races; insect-plant relationships.

RESUMO. Análise da diversidade genética de *Metopolophium dirhodum* (Walker) (Hemiptera, Aphididae) por meio de marcadores RAPD. A emergência de raças hospedeiro-especialistas em afídeos pode constituir um obstáculo ao manejo de pragas por meio de plantas resistentes. Existem exemplos de raças hospedeiro-especialistas em afídeos de cereais, embora a ocorrência de raça hospedeiro-especialista no pulgão-verde-pálido-do-trigo *Metopolophium dirhodum* (Walker, 1849) (Hemiptera, Aphididae) não tenha sido relatada ainda. Marcadores RAPD foram utilizados para avaliar os efeitos da distância geográfica e do hospedeiro sobre a diversidade genética de linhas clonais de *M. dirhodum*. Vinte e três clones foram coletados em aveia e trigo em doze localidades do sul do Brasil. De vinte e sete iniciadores usados para a análise, apenas quatro iniciadores mostraram polimorfismos. A análise de agrupamento por similaridade genética revelou haver quatorze genótipos, cinco dos quais coletados exclusivamente em trigo, sete exclusivamente em aveia e dois em ambos hospedeiros. Não houve correlação entre as similaridades genéticas e a distância geográfica. A análise da variância molecular demonstrou que alguns marcadores RAPD não se distribuem aleatoriamente entre as linhagens clonais coletadas em aveia e em trigo. Estes resultados sugerem a existência de raças hospedeiro-especialistas em *M. dirhodum* no Brasil, hipótese esta que deve ser investigada combinando-se dados ecológicos e genéticos.

PALAVRAS-CHAVE. Diversidade genética; interação inseto-planta; pulgão-amarelo-do-trigo; raças hospedeiro-especialistas.

Metopolophium dirhodum (Walker, 1849) is an important pest in cereals because of its damage on plants by sap suction and phytoviruses transmission. Poaceae are the host-plants of *M. dirhodum* in its asexual phase and this aphid is more often found in crops such as wheat, barley, rye, oats and maize (Dean 1974; Nicol *et al.* 1997). The presence of this aphid in South America was first recorded in 1970s in Chile and Brazil, when it became the major pest of wheat (Zuñiga 1986, Salvador 1999). In Brazil, *M. dirhodum* is more abundant in southern regions (Salvadori & Tonet 2001) and there has not been any record of the occurrence of sexual phase (Lopes-da-Silva, personal information).

Fewer studies on the genetic diversity of *M. dirhodum* have been done compared to other cereals aphids worldwide. At molecular level, De Barro *et al.* (1995) did not found any association between genetic polymorphisms and host preference in this aphid in southern England. Nicol *et al.*(1997) found smaller genetic variability on recently introduced New Zealand populations of *M. dirhodum* than on Scotland populations of *M. dirhodum*. On other hand, in cytogenetics studies, Rubin-de-Celis *et al.* (1997) reported the presence of two karyotypes within this aphid in southern Brazil: 2n=16 and 2n=18, suggesting an important genetic divergence caused by chromosomal arrangements.

The genetic diversity in aphids populations within a geographical region could be a result from natural selection imposed by hosts and from population division by migration (Loxdale 1990, De Barro *et al.*1997). Despite the high level of gene flow observed in some aphids, which prevent occurrence of geographically isolated populations in contiguous areas (Loxdale 1990), genetic structuring at micro geographical scale (< 1 km) has been found in *Metopolophium dirhodum* (De Barro *et al.* 1995). However, host plant is considered the key factor responsible for genetic differences among sympatric aphid populations (Lushai *et al.* 2002). There are several reports of host-races within aphids that might be explained by selective pressures from host-plants (Diehl & Bush 1984). Plant secondary metabolites are important defense against insect

damage. Among them, phenolics have been described as major anti-herbivore compounds (Urbanska *et al.* 2002). Hydroxamic acids are the most important substance that promotes plant resistance by antibiosis against aphids (Givovich & Niemeyer 1994). High level of these compounds are found in wheat and maize but are absent in oats and barley (Figueroa *et al.* 2002). The differential selective pressure caused by hosts with presence or absence of hydroxamic acids on the genetic composition of cereals aphids populations is still unknown (Figueroa *et al.* 2002).

Using RAPD markers to characterize twenty-three clonal lineages of *M. dirhodum* collected in different localities in southern Brazil, we evaluated the effect of host and geographical distance on the genetic diversity of this important cereal aphid.

MATERIAL AND METHODS

Field Sampling. Twenty-three *M. dirhodum* clones were collected in wheat and oat fields in Paraná (PR) and Rio Grande do Sul (RS) (Table I). Field collects were performed from July to September (2001 and 2002).Clones from same locality were collected in the same crop field . A single individual from each field was used to establish a clonal lineage (putative clones) in greenhouse. For this, the samples were raised on wheat seedlings planted in 4 L pots protected by plastic tubes with their tops covered by a voil fabric in order to avoid parasitism by microhymenopterous.

DNA extraction. The DNA was extracted from five specimens from the each clonal lineage using Carvalho & Vieira (2001) protocol with some modifications. The insect bodies were grounded manually in 60 µL buffer extraction (200mM Tris-HCl pH 8.0; 2 M NaCl; 70mM EDTA pH 8.0) and 15 µL of sarcosyl (5%) was added. After incubation for 30 min at 65°C with occasional mixing, the extract was centrifuged at 10000 rpm for 15 min and the supernatant recovered to a new microtube. DNA was precipited by adding 110 µL of ammonium acetate (10 M) and 250 µL of cold isopropanol to the aqueous supernatant. The solution was left overnight at –20°C and centrifuged for 15 min at 10000 rpm. The pellet was washed with 70 % ethanol, air, dried resuspended in 25 µL of TE buffer (10mM Tris-HCl, 1 mM EDTA pH 8.0) containing RNase (10mg/ml), and stored at –20°C. DNA concentration was estimated by fluorescence using DyNA Quant 200 minifluorimeter (Hoefer Instruments).

RAPD reactions. PCR for RAPD amplification were performed in 25 µL aliquots containing approximately 25 ng genomic DNA, l x PCR buffer[20mM Tris HCl (pH 8,4), 50mM de KCl] 3.0 mM $MgCl_2$, 100 ìM of each dNTP, 0.5 ìM of primer and 1 U de *Taq* DNA polymerase. The amplifications were performed in a PTC-100™ (MJ Research, Inc.) using the following temperature program: 5 min at 94ºC followed by 40 cycles of 1 min at 94ºC, 90 s at 40º C and 2 min at 72 °C, with a final extension of 5 min at 72 °C. Amplification products were analyzed by electrophoresis at 5V/cm in agarose gel (1.5 %). The gel was stained with ethidium bromide solution (1.0 mg/ml) and photographed with KODAK EDAS 120 system.

Table I. Host-plants, localities, number and code for *Metopolophium dirhodum* clonal lineages collected on wheat and oats.

Host-plant	Localities	Number of clones	Code
Wheat	Londrina-PR	3	W1
			W2
			W3
	Ponta Grossa-PR	2	W4
			W5
	Ijuí-RS	2	W6
			W7
	Tapera-RS	1	W8
	Cruz Alta-RS	1	W9
Oats	Campo Mourão-PR	1	O1
	Ijuí-RS	1	O2
	Cruz Alta-RS	3	O3
			O4
			O5
	Panambi-RS	3	O6
			O7
			O8
	Carazinho-RS	1	O9
	Passo Fundo-RS	1	O10
	Não-Me-Toque-RS	2	O11
			O12
	Ibirubá-RS	1	O13
	Selbach-RS	1	O14

Data Analysis. Only RAPD fragments (bands) less than 2.5 kb and reproducible in two or more gels were scored and considered for the analysis. A matrix of Jaccard's distances (Sneath & Sokal 1973) were estimated between all pairs of twenty-three clonal lineages as follows: S= a/(a+b+c) where: **a** is the number of bands present for both clonal lineages; **b,** the number of bands present for a clonal lineage1 but not for clonal lineage 2 and **c** the number of bands present for the clonal lineage 2, but not for clonal lineage 1. The clustering procedure UPGMA – Unweighted Pair Group with Arithmetic Mean (Sneath & Sokal 1973) was used to study the genetic relationships among the clones based upon distance matrix using NTSys 2.0 software (Rohlf 1998).

The genetic isolation by distance hypothesis was tested using Mantel test to correlate matrices of the genetic dissimilarity and geographic distance. Dissimilarity was obtained by the formula $DS_{ab} = 1\text{-}S_{ab}$, where DS_{ab} is dissimilarity index between a and b and S_{ab} is Jaccard's Similarity index between a and b. To avoid the confounding effect of host and geographical distance on genetic diversity, the Mantel test was performed separately for clonal lineage according to the host.

A two-level Analysis of Molecular Variance (AMOVA) was performed to investigate the contribution of host-plant to the total genetic diversity (clonal lineage within hosts). AMOVA-PREP (Miller 1998) and WINAMOVA 1.55 version (Excoffier *et al*., 1992) were used in this analysis.

RESULTS AND DISCUSSION

From the 27 RAPD primers tested, only four (OPA-2, OPA-3, OPA-4, OPA-7) showed clearly amplified DNA bands that were selected for analysis (Table II). A dendrogram is presented in Fig.1, where fourteen RAPD genotypes could be distinguished from the twenty-three clonal lineages. Five genotypes were observed exclusively in wheat; seven exclusively in oats and two were observed in both plants species. Considering the few RAPD markers obtained, these results revealed an unexpected high genetic diversity for a introduced parthenogenetic organism. This finding could be explained by variation of number of chromosomes set found in *M. dirhodum* specimens collected in Brazil (Rubin-de-Celis *et al*.1997). Different chromosome number within *Rhopalosiphum maidis* (Fitch) has been associated with host preference (Blackman *et al.*1990).

The association between genetic dissimilarity and geographic distance was r =0.27 (p = 0.08) for clonal populations collected on wheat and r= -0.17 (p=0.13) for clonal lineages collected on oats. This indicated that spatial and genetic distances are not correlated and high genetic similarity between lineages is not a direct consequence of proximity between collection sites, which suggests absence of genetic isolation by distance, at least at macro-geographical scale. In Europe, aphids have high gene flow, which is a barrier to genetic structure formation by geographic isolation. (Loxdale 1990). However, De Barro *et al*. (1995) found genetic structure at micro geographic level and absence of association between genotypes and hosts for *M. dirhodum* in southern England. This surprising finding is related with occurrence of sexual phase in this region, which sexual recombination prevents disruptive selection effects caused by hosts (De Barro *et al*. 1995). On other hand, in New Zealand (as in Brazil), *M. dirhodum* has no sexual phase and there is no genetic structure at micro geographic level. (Nicol *et al.*1997).

The AMOVA analysis (Φ_{st} = 0.21 p< 0.001) indicated that there are significant differences between RAPD markers frequencies of clonal populations collected from wheat and oats. The genetic difference based on host-plant is considered the first criterion for host-race definition (Drès & Mallet 2002). However, undoubted host-race identification depends on measuring performance and choice of the insects, reciprocally, in experiments for host-adaptation assessment (Lushai *et al*. 2002).

Table II. RAPD polymorphisms found within 23 clonal lineages of *Metopolophium dirhodum* using a set of four primers.

Primer	Total of Reproducible RAPD markers	Number of RAPD polymorphic markers	% de polymorphic markers
OPA-2	7	3	42,8 %
OPA-3	9	2	22,2 %
OPA-4	7	1	14,2 %
OPA-7	7	4	57,1 %
Total	30	10	33,3 %

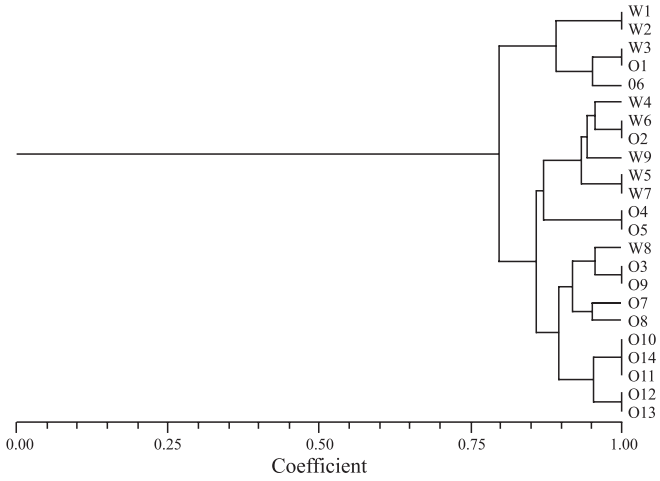

Fig.1. Dendrogram of genetic relationships among clonal populations of *Metopolophium dirhodum* using Jaccard Similarity Index (value 1.00 on x-axis represents 100 % of similarity). Codes for clonal lineages according to Table I.

Among RAPD polymorphisms, we found a strong association of 1.200 bp marker obtained with OPA-4 primer and host (Fig.2). This marker was found in eleven out fourteen lineages collected on oats and only in one lineage collected from wheat.

Sunnucks *et al* (1997) also reported an almost perfect correlation between RAPD markers and host plants in studies with *Therioaphis trifolii* (Monell,1882). This 1200 bp may be only associated to a putative *M. dirhodum* "oat-race". The occurrence of a single clonal lineage in wheat could be result from random landing of alatae colonizers. Thus, aphids could be found on inadequate plant in early stages of host-colonization (Hartworne & Via 2001).

Within cereals aphids, *Schizaphis graminum* (Rondani, 1852) presents populations characterized in different biotypes, which are considered as highly divergent host-races (Anstead *et al.* 2002; Lopes-da-Silva *et al.* 2004) or complex species (Wilson *et al.* 2003). More recently, it has been proposed that

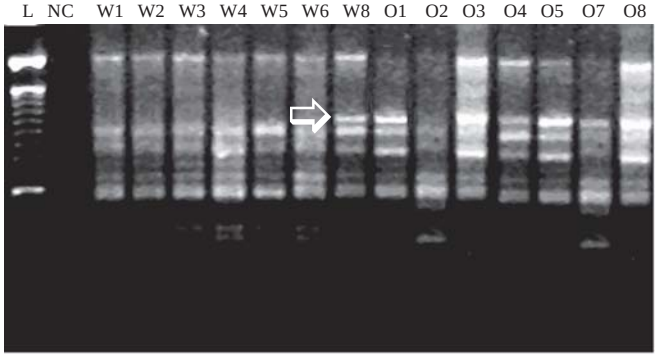

Fig. 2. RAPD profile of *Metopolophium dirhodum* clones obtained with use OPA-4 primer. White arrow is showing the marker (1200bp) more found in clonal lineages collected in oats (codes according to table I).

Sitobion avenae has two host-races: "cocksfoot-race" and "wheat-race" those are genetically and ecological distinct (Lushai et al. 2002). Also, the population of this latter aphid is more genetically variable on wheat than on oats, which was attributed to the mutagenic effects of hydroxamic acids contained within wheat (Figueroa et al. 2002). Regarding M. *dirhodum*, there is no description of host-races within populations of this aphid.

Selection by the host plant better explains genetic differences among clonal lineages of *M. dirhodum* than geographical distances. Thus, we suggest that all or part of genetic differentiation within clonal lineages of this aphid could be related to the adaptation to the host-plant. The role of hyrdoxamic acids (present in wheat and absent from oat) as natural selection agent should be investigated in experiments comparing performance of clonal lineages genetically characterized, in both hosts. To our knowledge, this is the first report regarding the appraisal of the genetic variability of South American populations of *Metopolophium dirhodum* based on host.

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