

# Analysis of Microsatellite Markers for Population Genetic Studies of the Chagas Disease Vector, *Triatoma dimidiata*

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## Summary

Eight published microsatellite primer sets were tested on six populations of *Triatoma dimidiata* from Guatemala. Three of the eight resulted in amplification useful for population genetic studies. In a preliminary analysis with these three primer sets, there appears to be genetic structuring in some populations of *Triatoma dimidiata* and evidence of migration among populations. The use of microsatellite markers to address questions of population structure of *T. dimidiata* will require development of additional loci.

## Introduction

Hematophagus Triatomine vectors carry the parasite that causes Chagas disease and control of these vectors is the management of choice. *Triatoma dimidiata* presents a challenge to control of Chagas in Central America due to its widespread distribution and large sylvan reservoirs. Knowledge of the population genetics of the Triatomines is critical for effective control.

Previous results using Randomly Amplified Polymorphic-PCR (RAPD-PCR) showed a high degree of genetic variation, low Nei's genetic distance, and Wright's fixation index ( $F_{ST}$ ) among domestic populations of *T. dimidiata* within one village and among adjacent villages up to 27 km apart, indicating that these populations are panmictic (Dorn et al., 2003). In contrast, populations from different states in Guatemala show a high degree of differentiation at the country-wide level and a moder-

ate degree of differentiation within sylvan or domestic populations (Calderón et al., 2004). By morphometric studies of head characters, geographic distance appears to be more important than habitat (sylvan or domestic) in separating northern, middle and southern populations (Bustamante et al., 2004).

These results indicate that although the populations of *T. dimidiata* in Central America are closely related, there is clearly geographic structuring and some isolation by distance (by morphometry) and possibly habitat (by RAPD-PCR). It is yet to be determined whether geographic distance or habitat is more important in structuring populations. In addition, to better evaluate the risk of sylvan populations in transmission it is essential to know whether or not nearby sylvan and domestic populations are interbreeding. For effective control, the size of the panmictic unit in different endemic regions needs to be clarified. Good molecular markers could be quite valuable in answering these questions.

Recently, microsatellite markers were published for *T. dimidiata* (Anderson et al., 2002). These co-dominant markers have the advantage over the (mostly) dominant RAPD-PCR markers in that Hardy-Weinberg equilibrium may be tested in populations rather than assumed. This will allow for determination of significant inbreeding, selection and/or migration by testing for deviations from Hardy-Weinberg equilibrium in populations. In addition, the degree to which the larger population of *T. dimidiata* is subdivided into smaller populations can be estimated using  $F_{ST}$  or  $R_{ST}$  statistics. The variance in allele frequencies can be analyzed to see if the variance among the larger population can be accounted for by the variance among the smaller populations and the genetic distance among populations estimated to determine if this correlates with geographic distance or habitat.

## Materials and Methods

To test these microsatellite markers, one hundred fifty *T. dimidiata* vectors were collected inside houses from six villages (~30 bugs per village) between August 2001 and January 2002. The villages are located within 2-8 km of each other in the Guatemalan state of Jutiapa. DNA was isolated from the vector's legs (Dorn et al., 2003) and microsatellite sequences amplified from bugs (Anderson et al., 2002). Amplified products were electrophoresed on an ABI 377 automatic DNA Sequencer (Applied Biosystems, Foster City, CA) and the sizes of the microsatellite alleles determined using STRand software (Veterinary Genetics Lab, 1996-2000).

## Results and Conclusions

Of the eight published primer sets, two did not show any amplified

Table 1. Results with selected microsatellite loci for *T. dimidiata*

Primer set	reported allele sizes (Anderson et al., 2002)	observed allele sizes	No. of alleles (N=89)	% of individuals not showing amplification	Comments
TDMS 4	150-186	150-186	8	0%	Consistent amplification
TDMS 9	194-230	178-212	12	2.2%	Consistent amplification
TDMS 11	132-230	120-246	14	11%	Highly polymorphic, null alleles? Minisatellite?
TDMS 22	101-109	105-113	4	0%	Consistent amplification

product (TDMS 16 and 19), one showed inconsistent amplification (TDMS 1) and one primer set gave more than two bands per individual suggesting it may be recognizing a repeated sequence. The remaining four primer sets show consistent amplification, however, TDMS 11 was excluded from further study since a high number of individuals fail to show an amplified product (11%, table 1), which could indicate null alleles and underestimate levels of heterozygosity. The remaining three loci (TDMS 4, 9 and 22) were used to amplify individuals in the six populations. Two of the three microsatellite alleles were determined for all the 150 individuals in the six populations. For one locus, TDMS 9, we have data for three populations. These loci are highly polymorphic (4-12 alleles in 89 individuals, table 1) and no evidence of linkage disequilibrium was observed between microsatellite loci pairs ( $P = 0.112-0.353$ ).

In a preliminary data analysis of these alleles performed on GenePop (Raymond & Rousset, 1995), results show that three of the six populations are likely not in Hardy-Weinberg equilibrium and show a deficiency of heterozygotes whereas for the other three the opposite was found. In addition, there are allelic and genotypic distribution differences among all six populations ( $P=0$ ). There is a moderate genetic differentiation among populations ( $F_{ST} = 0.114$ ) and the number of mating migrants per generation, using the private allele method, showed an  $Nm = 3.5$ . These results suggest structuring in some populations of *T. dimidiata* in Guatemala and possibly that the size of the panmictic unit is different in different regions of Guatemala (comparing the RAPD-PCR results to these microsatellite results). There is evidence of migration among

populations indicating that effective control will require broad geographic coverage. Conclusive results about the genetic structure of *T. dimidiata* populations will require analysis of additional microsatellite loci.

Results here show that three of the published microsatellite markers for *T. dimidiata* are useful for population genetic analysis. However, successful application of microsatellite markers to questions of population structure of *T. dimidiata* will require development of additional markers.

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