Migration and Gene Flow Among Domestic Populations of the Chagas Insect Vector *Triatoma dimidiata* (Hemiptera: Reduviidae) Detected by Microsatellite Loci

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Presented by:

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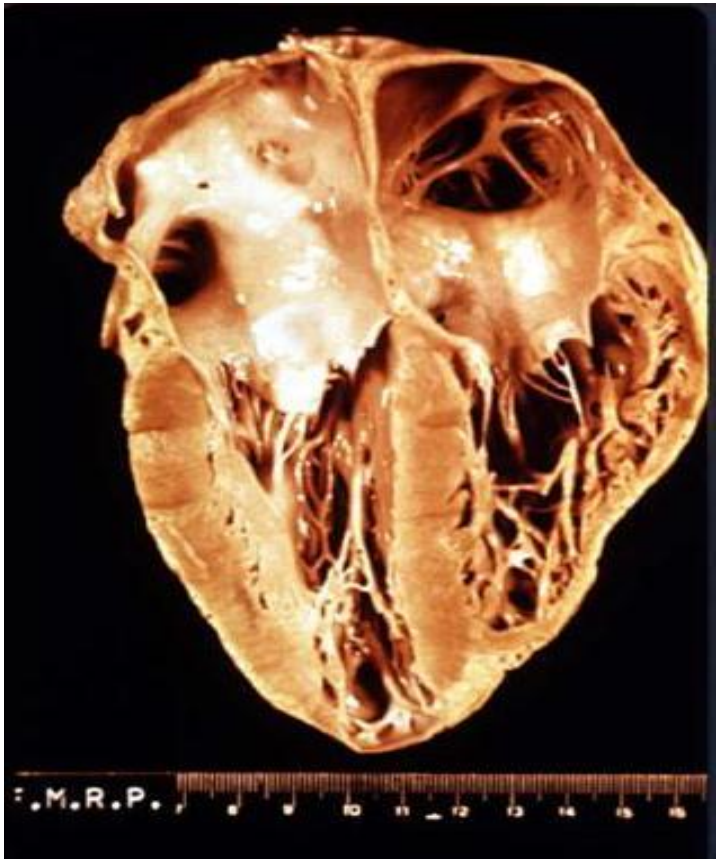
Triatoma dimitiata

- Blood sucking bugs of order Hemiptera and subfamily Triatominae
- Endemic in South America (Colombia, Venezuela, Ecuador, and Peru), Central America and Southern Mexico

Chagas disease

- Also known as American trypanosomiasis
- Tropical parasitic disease caused by protozoa *Trypanosoma cruzi*
- Spread mainly by Triatomine insects (kissing bugs)
- Also through blood transfusion, organ transplantation, eating food contaminated with the parasites and vertical transmission
- Early infections treatable with the medication benznidazole or nifurtimox
- Chronic disease leads to destruction of the nervous system, digestive system and heart

Chronic disease



Introduction

- Understanding migration and gene flow of insect vectors is critical to effective control and elimination of vector-borne diseases
- Tritomines transmit *Trypanosoma cruzi*, a causative agent of Chagas disease in humans
- Serious parasitic disease in Latin America
- The triatomine species that transmit *T. cruzi* differ in epidemiological importance
- Difference strongly influenced by vector movement
- Immature nymphs migrate, actively walking tens of meters

Introduction..

- Adults of some species known to fly up to 200 m-2 km
- Triatomines also migrate passively in wood, personal effects, agricultural products or by birds
- *T. dimidiata* varies morphologically, genetically and biochemically
- Shows variability in inhabiting domestic, peri-domestic and sylvatic habitats
- Has a tendency to colonize and recolonize houses-important for transmission

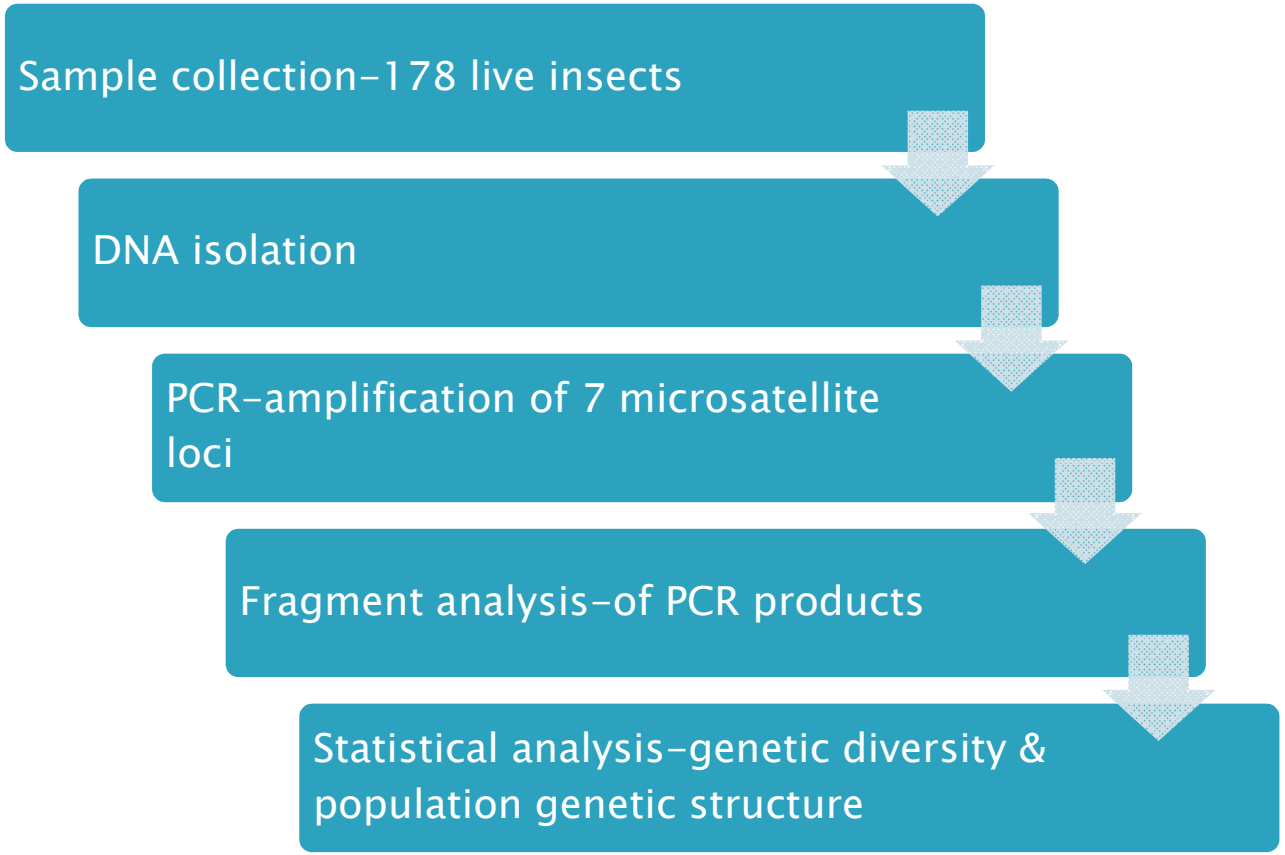
Introduction..

- Short sequence repeats of DNA
- Microsatellite loci are valuable markers in genetic structure and vector population studies
- Loci are highly variable-differ in number of repeats due to polymerase slippage during replication
- Large number of alleles allows fine-scale discrimination of closely related individuals, providing the statistical power to understand gene flow and the genetic structure of populations
- Reproducible, codominant, PCR-based, most are noncoding, hence neutral markers

Objective

- To understand migration and gene flow of *T. dimidiata,* and gain a better picture of the vectors ecology
- Using microsatellite loci to analyze the population genetic structure and gene flow

Materials and methods



Materials and methods..

- Study area-6 villages in southern Guatemala (Latin America) where Chagas disease is hyperendemic
- Sample collection-178 live T. dimitiata specimens were examined, collected from houses
- Legs removed-preserved in 95% alcohol & 5% glycerol and stored at -20°C
- DNA isolation-using published methods

PCR

- Amplification of 7 microsatellite loci using a Techne Thermocycler
- 95°C for15 min, followed by 35 cycles of 94°C for 30s, 52°C for 90s, 72°C for 60s and a final extension at 72°C for 10 min using 200ng of DNA and fluorescent dye
- Sizes of the fluorescently labeled PCR products were determined using either an Applied BioSystems 3100-Avant Genetic Analyzer or Applied BioSystems 3730xl DNA Analyzer
- Data recorded in .fsa format data files
- Fragment Analysis-fsa data files viewed using GeneMapper version 3.7 (Applied Biosystems)
- Identify and classify the alleles present per locus and per insect

Statistical analysis

- Summary statistics for each locus and village
- Locus-allele size range, average number of alleles per village and percent of individuals with missing data
- Village-genetic diversity estimated using Nei's unbiased estimator of HS and unbiased allelic richness using FSTAT
- Estimating population genetic structure
- Two approaches used:-
- First, villages and houses considered as discrete physical units
- Multilocus versions of Wright's *F* statistics used in a hierarchical AMOVA to test for significant population structure among villages (FRT) and among houses within villages (FSR)

Statistical analysis..

- Second approach used Bayesian Markov chain Monte Carlo (MCMC) simulation to estimate the number of genetic clusters and geographic distribution of the genetic clusters among villages and among houses within a village
- Software STRUCTURE version 2.3.2 used
- Distribution of genetic clusters within and among villages visualized using CLUMPP ver. 1.1.2 and DISTRUCT ver. 1.1
- Test for biased dispersal and variation in infection prevalence
- Biased dispersal between males and females and between *T. cruzi* infected and uninfected insects-FSTAT Version 2.9.3.2
- Nested ANOVA used to test difference in prevalence of infection of insect with *T. cruzi* among houses within and among villages

Results

- Summary statistics for each locus and village
- All seven microsatellite loci were highly polymorphic;6-14 alleles per locus, per village
- Genetic variability of loci quite similar among the six villages; allelic richness varies from 9-11 and Nei's HS ranges from 0.74 to 0.85

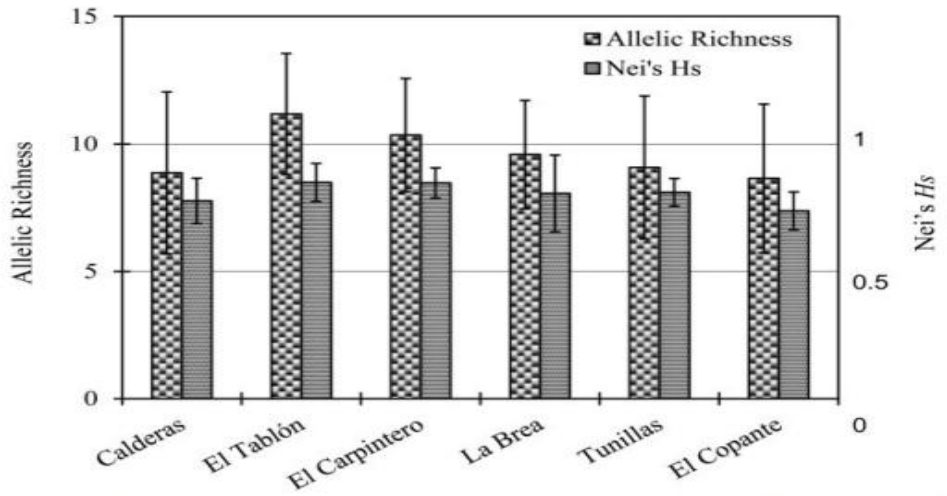


Fig. 2. Similarity of the genetic variability of seven microsatellite loci in *T. dimidiata* from six villages in Jutiapa, Guatemala, based on allelic richness and Nei's H_S.

Estimating Population Genetic Structure

- Tested related genetic differentiation and relatedness
- Hierarchical AMOVA indicates small but statistically significant differences among villages (FRT=0.049) and among houses within villages (FSR=0.110)
- Bayesian MCMC simulations from STRUCTURE found five genetic clusters
- Migration of *T. dimidiata* is inferred–all the clusters are found in multiple villages and all villages have more than one cluster
- Multiple clusters per house indicated migration and gene flow among houses and among villages

Genetic structure & Relatedness

Table 3. Hierarchical AMOVA shows significant population structure among villages (F_{RT}) and among houses within villages (F_{SR})

Source of variation	df	Sum of squares	Variance components	Fixation Index	*P*
Among villages (R)	5	53.16	0.13	$F_{RT}=0.049$	< 0.001
Among houses within villages (S)	24	127.30	0.29	$F_{SR}=0.110$	< 0.001
Total	213	582.98	2.77		

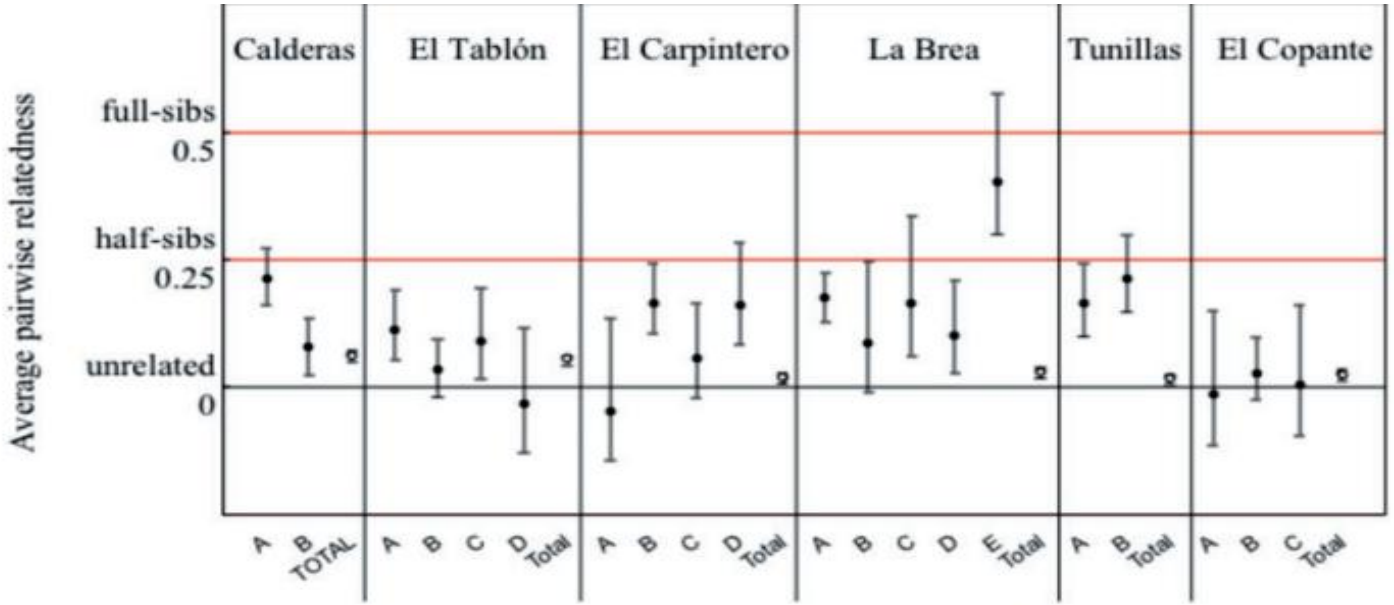


Fig. 3. Variability in average pairwise relatedness among houses with >2 insects (A–E,•) and for the 28–30 insects from each village (Village, ○). Values are ± SEM. Note values can be negative due to sampling error.

Genetic differentiation

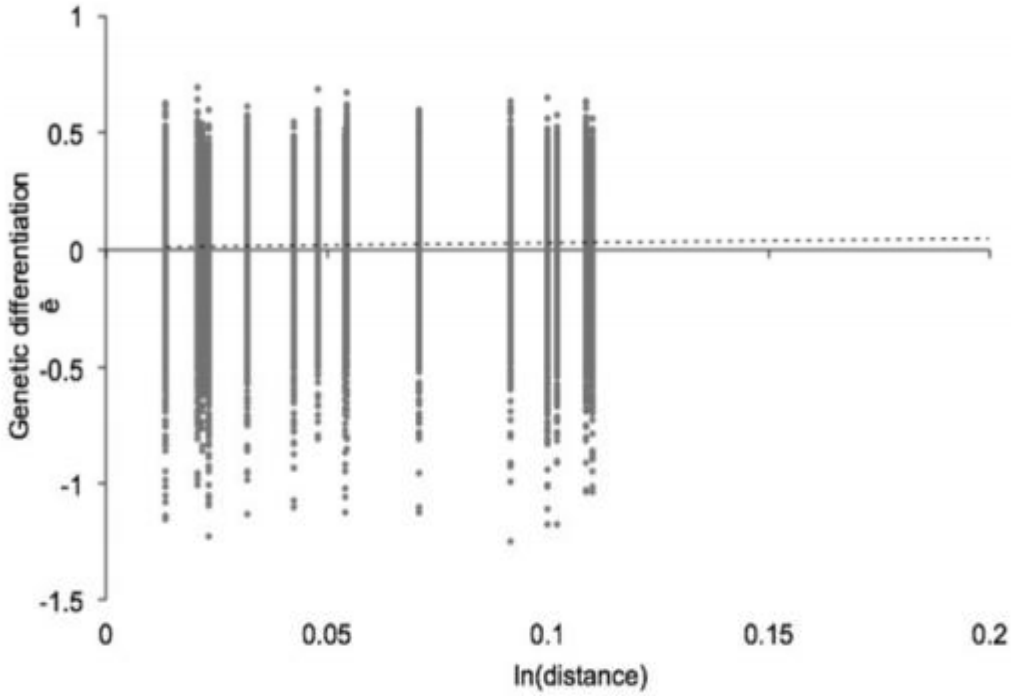


Fig. 4. Linear regression (. . . . . .) showing that genetic differentiation between insects increases with geographical distance. Data are pairwise genetic distances among six villages (28–30 insects per village. Linear regression: $\hat{e} = 0.0719 + 0.0165 * \ln(\text{distance})$; 95% confidence interval for slope [0.0061, 0.292]).

Genetic clusters

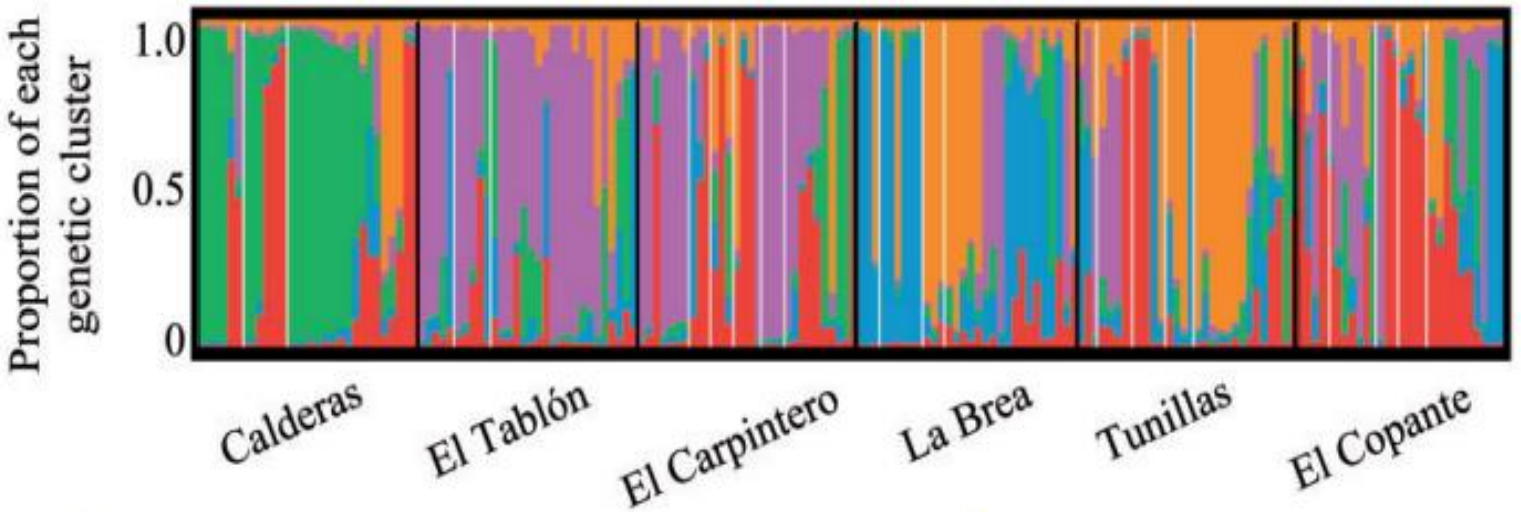


Fig. 5. Distribution of five genetic clusters (indicated by colors) among villages, based on Bayesian MCMC simulation. Vertical black lines separate the six villages, indicated below the figure. White lines separate the houses. Each insect is represented by a thin vertical line partitioned into colored segments indicating one of K = 5 genetic clusters. For example, the first house in Calderas contained four insects from the green cluster, an orange–green–blue admixture, and an orange–purple admixture. Only houses with more than two insects are delineated; the remaining insects are pooled in the rightmost partition for each village.

Testing Biased Dispersal and Variation in Infection Prevalence

- No evidence of biased dispersal based on *T.cruzi* infection status (uninfected vs infected: FST 0.08 vs 0.07, P>0.05) or sex (female vs male:FST 0.07 vs 0.05, P>0.05)
- No difference in prevalence of infection of insects with *T. cruzi* in houses within and among villages (P>0.05)

Discussion

- Population of *T. dimitiata* contain distinct genetic clusters found in multiple houses within the same village
- Suggests migration and gene flow among houses
- Tests for biased dispersal indicates migration is not influenced by *T. Cruzi* infection nor sex
- All six villages had similar estimates of allelic diversity showing no dramatic difference in factors that affect allele frequencies and distribution, including *T. dimidiata* population size and gene flow
- Generally, *T. dimitiata* is a mobile vector

Conclusion–Control implications

- Results on the population genetic structure of *T. dimidiata* illuminates the role of migration and gene flow in Chagas disease transmission and provide important information for the design of effective control strategies
- Pattern of genetic variability detected suggests use of insecticides alone is not a sustainable control method
- Reinfestation is possible due to migration
- Alternative strategies–house improvements, using screens and mosquito nets and reducing the amount of clutter
- Necessary to create a more effective barrier and reduce infestation rates

Critique

- Comparing two studies with different protocols and study areas making the developmental process hard to follow


THANK YOU...