University of Vermont

Rubenstein School of Environmental and Natural Resources

Department of Natural Resources

PhD DISSERTATION PROPOSAL

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Assessing Source-Sink Dynamics of the Chagas Disease Vector, *Triatoma dimidiata,* in high-risk communities in Guatemala

By

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Project Summary

This research aims to understand the small-scale distribution and dispersal of *Triatoma dimidiata*, a vector of Chagas disease, within two towns from Jutiapa, Guatemala. *Triatoma dimidiata* is the main vector of the protozoan parasite *Trypanosoma cruzi* in Central America, where the number of communities at risk of infection is steadily increasing. Currently, most control efforts focus on eliminating the vector from households through the use of insecticides; however, *T. dimidiata* is capable of living in domestic, peridomestic and sylvatic environments. The variation of quality among domestic and sylvatic habitats may allow populations to behave as a source-sink system, where sylvatic habitats are reservoirs, peridomestic environments are ecological bridges, and domestic households are colonization sinks. Consistent with this hypothesis, studies show that *T. dimidiata* is likely to re-infest sprayed houses within the same year of treatment.

I will integrate the use of high-throughput genetic data and geospatial tools to test whether source-sink dynamics can explain spatial and temporal patterns of infestation within the towns of El Chaperno and El Carrizal in Guatemala. To understand the spatial movement patterns of the vector, I will quantify genetic relatedness of individuals within each town and detect any clustering patterns that can indicate domestic colonization. To assess the relative importance of external migration versus local colonization, I will look at the population genetics of re-infesting populations after seasonal migration and pesticide fumigation. This work will increase the understanding of source-sink dynamics of *T. dimidiata*, assess the impact of migrants in domiciliary environments, and quantify the effectiveness of fumigation in vector populations in Guatemala.

I. Conceptual Framework

a. Overall Goal

My work is part of an interdisciplinary collaboration that aims to increase the understanding of Chagas disease transmission in high-risk, rural communities. Within this context, I will integrate the use of high-throughput sequencing data and geospatial tools to (1) obtain genetic information from *Triatoma dimidiata*, *Trypanosoma cruzi*, and hindgut micro-biota; (2) Analyze multiple geographical scales using single nucleotide polymorphic markers (SNPs) generated from samples across Central America; (3) Assess relatedness between individuals as an indicator of connectivity among houses within El Chaperno and El Carrizal; and (4) assess the patterns of re-infestation after a dry season in El Chaperno and after insecticide fumigation in El Carrizal.

b. Specific objectives

i. **Characterize a wide range of taxa from the abdomen of *Triatoma dimidiata,* and assess the geographical scales at which SNP markers can be employed to infer genetic relatedness.**

The specific tasks of these objectives are:

1. Obtain information from a wide range of taxa from the abdomen of *T. dimidiata* using a single method of high-throughput sequencing. This will be a metagenomics-like project were I will obtain a comprehensive set of SNP markers from *T. dimidiata* and *T. cruzi*; as well as generate information from blood meal sources and micro-biota from the insect's hindgut. Although findings of all this data will be reported on my first paper, I will specifically work with *T. dimidiata* data once the pipeline has been refined.
2. Assess the geographical scales at which a high-throughput set of SNP markers from *T. dimidiata* can yield information to infer genetic relatedness among individuals. For this task I will look at three different scales: across countries of Central America, within Guatemala, and within three small-scale regions of Guatemala and El Salvador.
3. Assess the spatial connectivity of the individuals across and within countries of Central America. I will use genetic relatedness between individuals as an indicator of spatial connectivity. To accomplish this task I will parameterize/collapse SNP data into geographically informative data to develop semi-variograms of genetic relatedness. One of the bigger challenges of this objective is to find a way to collapse genotypic data into a variable that explains genetic distance analogous to the geographical distance of the individuals.

ii. **Testing source-sink dynamics of *Triatoma dimidiata* across households in two towns of Jutiapa, Guatemala.**

The aim of this objective is to understand the temporal and spatial distribution patterns of *T. dimidiata* at a town level. I will use genetic data to infer house colonization, movement of the vector between houses, events of isolation, and re-colonization of *T. dimidiata* within towns. To assess this objective we developed a sampling scheme that will allow me to tackle the following questions:

1. What are the spatial relatedness, house clustering patterns and genetic diversity of *T. dimidiata* within El Carrizal and El Chaperno? For this question I hypothesize that there is (1) genetic clustering in houses that are spatially isolated, and (2) houses categorized as most at-risk conditions provide a suitable environmental for colonization. These houses eventually become reservoir of *T. dimidiata*, which will be detectable by assessing genetic relatedness of the insects in these houses to insects in households at close proximity.
2. How does the structure of the population of *T. dimidiata* changes after a dry season? Can we detect sink dynamics within our populations after a dry season drives insects to disperse into domestic environments and out of sylvatic or peridomestic habitats? I will assess genetic structure based on seasonal changes in El Chaperno. I hypothesize that dispersal events predicted during dry season in El Chaperno will increase genetic diversity within the town, therefore diluting any genetic clustering detected earlier

during the rainy season. Furthermore I predict that during dry the density of populations inside houses is sustained by the movement of insect into the house rather than by reproduction of the insects inside the house.

3. How does the structure of the population of *T. dimidiata* changes after insecticide spraying? In El Carrizal I predict that, after spraying, most of the re-introduction of insects happens at the edge of the town or close to highly vegetative areas. I also expect to see an increase of genetic diversity since insects collected after spraying will be migrants from various sylvatic patches. If application of the insecticide is not effective, then I predict stronger clustering within the town, with no relation to surrounding vegetation or edges.

II. Background

a. Chagas Disease

Chagas disease is endemic to Latin American, with an estimated 8 million people infected (Organización Panamericana de la Salud, 2006; Figure 1). Approximately 30-40% of people with Chagas disease eventually develop chronic Chagas, living approximately 10 to 30 years before they die of symptoms associated with enlarged heart, colon, and/or esophagus (Rassi, e*t al.* 2010 and WHO, 2002). There is no vaccine to prevent Chagas disease, and most prevention efforts focus on the management of households either by insecticide spraying or house improvement (Monroy *et al*. 2009 and WHO, 2002).

Chagas disease is caused by *Trypanosoma cruzi*, a protozoan parasite transmitted by the insect family Reduviidae. About 130 reduviids can potentially transmit the disease; but only five species, *Triatoma infestans, Rhodnius prolixus*, *Panstrongylus megistus*, *Triatoma brasiliensis*, and *Triatoma dimidiata*, are known to infect humans (Monroy *et al*. 2009 and WHO, 2002). My research will focus on *T. dimidiata*, the primary vector of Chagas disease in Guatemala and an important vector in the rest of Central America (Figure 1).

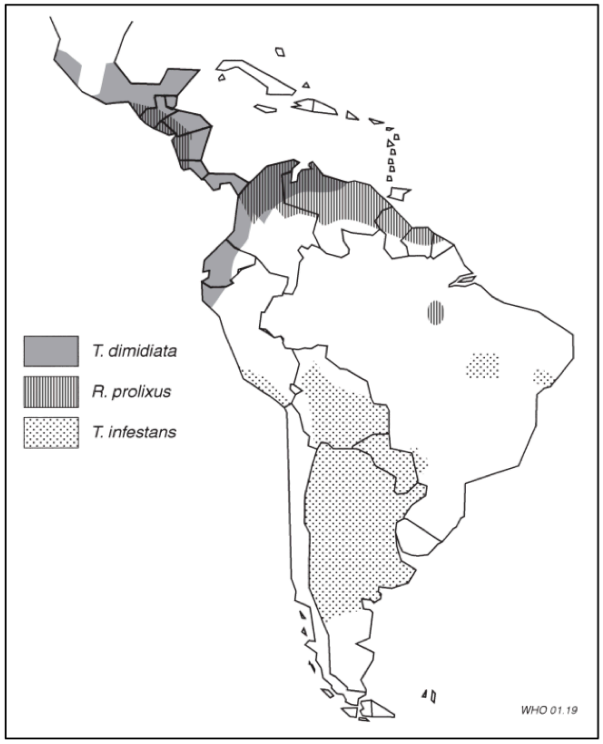

Figure 1. WHO, 2002. The geographic distribution across 21 Latin American countries of three principal vectors of Chagas disease.

b. Life History

T. dimidiata takes a mean of 11 months to fully develop as an adult. Once it has reached a reproductive stage it lives between one to three years (Zeledón, *et al*.1970). It has five nymph stages and an adult stage, all of which feed from mammalian or bird blood (Zeledón, *et al*.1970; Figure 2). *T. dimidiata* females with constant access to blood meals lay ~1,300 eggs over their lifetime, laying a clutch of aproximately 31 eggs per day (Zeledon *et al.* 1970). Females mate an everage of 13 times during their adult life, but mate only once for each copulation event, and preferably after a bloodmeal (Vargas, *et al.* 1985 and Zeledón *et al.* 1970). It has been shown that both males and females will repeatedly mate with the same or closest partners available to avoid environmental exposure; however, this trend only occurs when blood source, shelter and mates are available (Martinez-Ibarra, *et al.* 2001, Payet *et al*. 2009 and Zeledón *et al*. 1970). It has been reported that females can lay fertile eggs several months after the last copulation, but little is known about the paternity distribution within each egg clutch (Zeledon *et al.* 1970).

c. Population Ecology of *Triatoma dimidiata*

Unlike other Chagas vectors, *T. dimidiata* is most commonly found at low population densities in domestic environments, going through a seasonal

depletion during raining season and an increase population size during dry season when the food sources in sylvatic habitats becomes scarce (Payet *et al*. 2009, Barbu *et al*. 2009 and Gourbière *et al*. 2008, Monroy, *et at*. 2003). Dispersal of *T. dimidiata* into houses happens from short distances since *T. dimidiata* is a poor flyer, and most of its movement is terrestrial or by hitchhike on a host (Dumonteil, *et al*. 2004 and Monroy, *et al*. 2003). This vector is opportunistic, and moves primarily to feed and mate. Its dispersal is non-directional, given that it does not return to its original site of birth or reproduction (Dumonteil, *et al.* 2004 and Zeledón, *et al*. 1970).

Triatoma dimidiata populations behave as typical source-sink dynamic systems, where outdoor habitats are the sources and houses are sinks (Barbu *et al*. 2009). A previous study in the Yucatan Peninsula showed that houses are temporarily infested with up to a hundred insects during seasons of low-natural food sources (dry season); however, in rainy season, domestic populations decline to as low as 5 to 10 insects per household (Dumonteil *et al*. 2007). *Triatoma dimidiata* has a diverse habitat range, with the ability to remain in sylvatic ecosystems and feed on a wide variety of vertebrates ranging from mammals to amphibians (Zeledón *et al*. 1973, and Zeledón and Rabinovich, 1981). In domestic environments, if poor housing infrastructure and domestic animals provide food and refuge, a small number of individuals will likely establish and reproduce; however, data from the Yucatan Peninsula confirmed that 90 % of the individuals found in houses are migrants and not multigenerational settlers (Bustamante, *et al*. 2009, Dumonteil *et al*. 2007, Gourbière *et al*. 2008, Monroy, *et al*. 2011 and Payet *et al*. 2009).

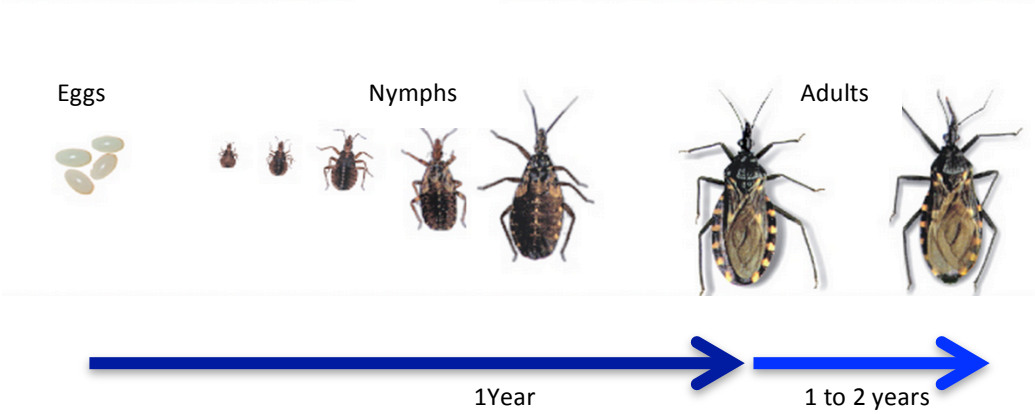

Figure 2. Life cycle of *Triatoma dimidiata* (open source).

d. Pesticide use in the context of *T. dimidiata* infestation

Infestation by non-domestic insects is emerging as a major challenge in controlling *T. dimidiata* (Barbu *et al*. 2009, Dumoteil *et al*. 2007). Previous studies have shown that the average time for a town to get re-infested after insecticide spray ranges from 3 to 6 months (Dumontiel, *et al*. 2004 and Monroy, *et al*. 2011). Conventional spraying has limited efficacy, so further

optimization of vector control strategies requires detailed knowledge of the changes in the vector population after houses have been treated. Previous studies have demonstrated that successful establishment of populations is driven by dispersal events that tend to occur in a gradient based on proximity to sylvatic sources (Gourbière *et al*. 2008 and Ramírez-Sierra *et al.* 2010; Figure 3). Ramírez-Sierra *et al.* (2010) inferred a spatial gradient of house infestation in four towns, where houses located at the periphery and closer to the surrounding bushes were at much more higher risk of infestation by non-domestic *T. dimidiata* (Figure 3). In our study I predict that we will see emerging clusters near highly vegetated areas, and that the insects within each cluster will not be closely related, but will come from different sylvatic or peridomestic sources.

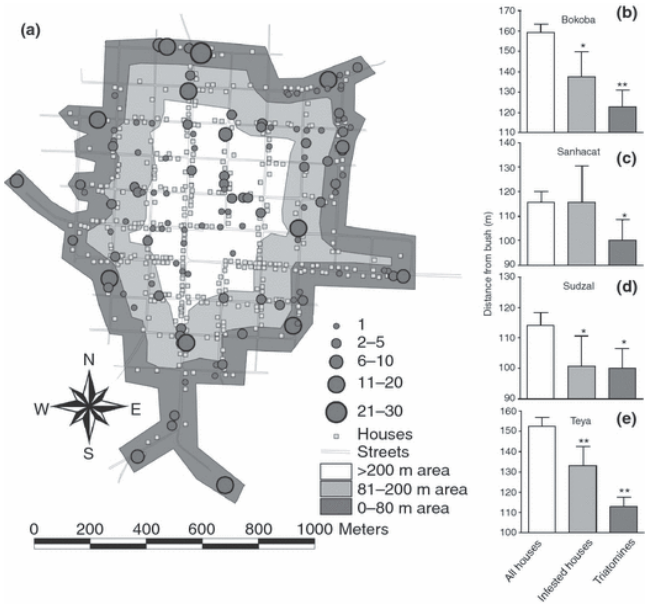

Figure 3. From Ramírez-Sierra *et al.* 2010. Circles indicate the cumulative *T. dimidiata* collections over a 2 year period, with the size proportional to the number of bugs. Shaded gray areas indicate village zones defined by the distance from the surrounding bushes.

III. Rationale and Significance

Vector-borne diseases, like Chagas disease, are a significant burden in limited resource areas, especially in developing countries. For this reason it is important to generate low-cost control programs in areas of high-risk. To generate such low-cost programs we need to work towards understanding the

population ecology and genetic diversity of both the parasite and vector at within-town resolution.

With the arrival of next-generation sequencing (NGS), there are new approaches to discover, sequence and genotype hundreds to thousands of markers across almost any genome of interest, even for organisms with scarce genome reference such as *T. dimidiata* (Davey, *et al*. 2011). These high-throughput data will enable us to do comprehensive genome-wide studies not only for *T. dimidiata*, but also in a wider scale of organisms, including *T. cruzi* and the vectors gut micro flora (the last two will be addressed by other members of our project).

Current genetic markers give limited information on the genetic diversity and movement pattern of *T. dimidiata*, but with the use of NGS tools we propose to overcome current limitations. I will provide genetic information on the movement of *T. dimidiata* within two towns from Jutiapa, Guatemala, and discuss the significance of using genetic relatedness to assess the genetic connectivity of individuals at different geographical scales.

Finally, this project's significance extends to a practical level: we will detect whether house can serve as reservoirs of the vector, and if source-sink dynamics are a driver to persistent events of infestation within a town. We will assess whether seasonal dispersal is a stronger component that reproduction for establishment of populations in houses. Finally, we will assess the effectiveness of fumigation by looking at the spatial patterns and genetics of the re-infesting population.

IV. Research approach

a. Project Description

i. **Characterize a wide range of taxa from the abdomen of *Triatoma dimidiata,* and assess the geographical scales at which SNP markers can be employed to infer genetic relatedness.**

Sample Data. We extracted DNA from 34 insects collected in a time span from 2006 to 2012. All samples were previously preserved in a mix of 90% ethanol and 5% glycerol, and stored at room temperature. Samples were sent to sequence to Floragenex® where Reduced Representation Library Sequencing (RAD-seq) data was generated for us.

Figure 4. Towns, regions and countries where samples were collected for the first objective of this study.

Study Area. We obtained samples from a wide geographical scale to capture as much genetic polymorphism as possible. Obtaining a wide polymorphic range allows us to capture a greater amount of markers, thereby increasing the resolution of our genetic data. We submitted 12 samples of surface sterilized legs to generate a genomic catalog for *T. dimidiata*, and 22 samples from insect abdomens to capture data from *T. dimidiata, T. cruzi*, blood meal sources and hindgut micro biota. Samples were collected from Belize, Guatemala, El Salvador and Nicaragua (Figure 4). Within Guatemala, eight towns from two regions, Jutiapa and Chiquimula, were sampled. In El Salvador 4 towns from the region of Santa Ana were sampled (Figure 4). All samples were obtained from the collection of the Laboratorio de Entomología Aplicada y Parasitología (LENAP) at Universidad de San Carlos de Guatemala.

Bioinformatics pipeline. We need a pipeline in order to sort information from an array of organisms that come from a single sample from *T. dimidiata*'s lower abdomen. To do this we first obtain raw data generated by Illumina high-seq® consisting of 100 bp long reads. Second, we map the reads to the *T. cruzi* reference genome. Then we generate a catalog of *T. dimidiata* leg samples, and map all samples to the catalog. Last, we BLAST all our samples to retrieve information from blood meal and biota (Figure 5).
From the sorted data we obtain from *T. dimidiata* and *T. cruzi*, we will obtain a set of SNP markers. Single Nucleotide Polymorphic markers come from the differences in a single base position across the genomic data of several organisms from multiple populations.

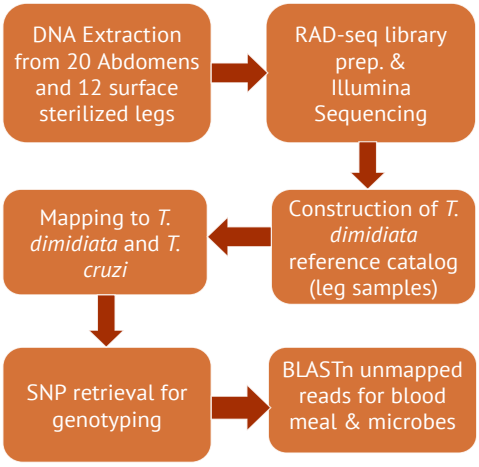

Figure 5. Workflow for DNA extraction, library preparing and bioinformatics pipeline for the project.

Data Analysis. I will generate maximum likelihood and Bayes trees to assess genetic distance among individuals. The trees will help answer the second objective, which is to parameterize the SNP data into distance measurements that can be analyzed using geostatic tools. We are still exploring the best type of data for this objective. John Hanley and I have found that the best match for analysis is to use patristic distance, and will use this method to measure the number of apormorphic step changes separating our samples. Patristic distance quantifies the amount of divergence of an organism from a common ancestor (Foument and Gibbs, 2006).

i. **Testing source-sink dynamics of *Triatoma dimidiata* across households in two towns of Jutiapa, Guatemala.**

Study area and collection periods. El Carrizal and El Chaperno in Jutiapa, Guatemala, were selected because of their reported high infestation rates with *T. dimidiata* (~30% annually), close geographic proximity, and distinct landscape in relation to one another.
To capture the effect of seasonal change in the *T. dimidiata* population due to seasonal effects, El Chaperno was samples before and after the dry season, since this is the time where most dispersal into town is know to happen (Figure 7). In order to capture the effectiveness of spray treatments, all houses from El Carrizal infested with *T. dimidiata* were sprayed with insecticide after the first visit. The second fieldwork did not occur until 6 months after all houses were sprayed to allow re-infestation of the town.
For each town we collected data at household level that includes: survey data (e. g. infrastructure condition, number of domestic animals), vector data (e.g. age and/or sex of the vectors, where were vectors found), and geo-positional data. All this information will be useful for geospatial analysis were I will be able to correlate genetic relatedness to factors such as infrastructure, house isolation, clutter, and presence of domestic animals across each town scale.

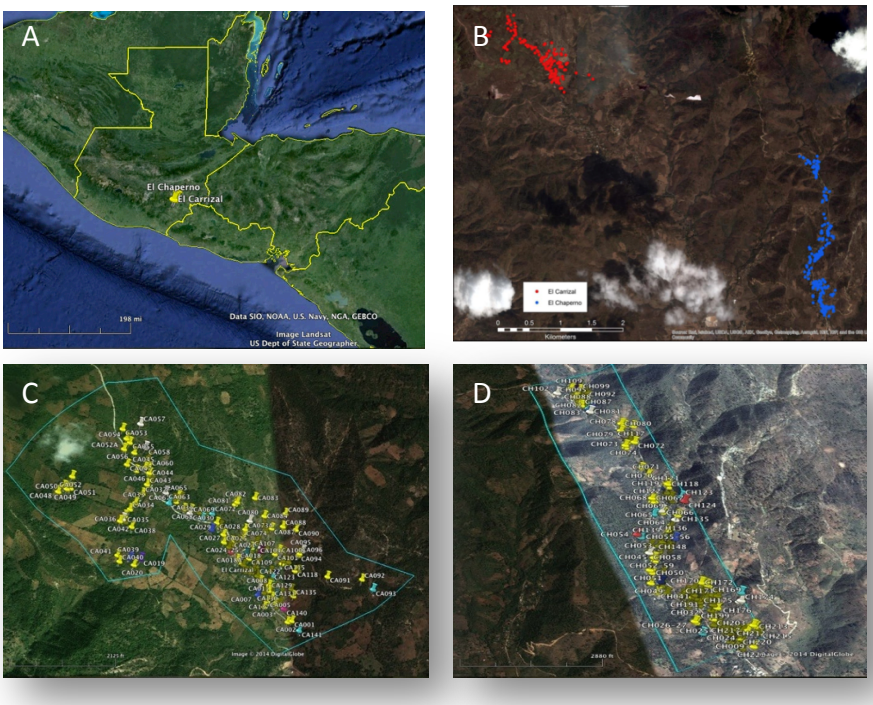

Figure 6. (a) Position of El Carrizal and El Chaperno within Guatemala; (b) Distance and topography separating both towns; (c) El Carrizal; (d) El Chaperno

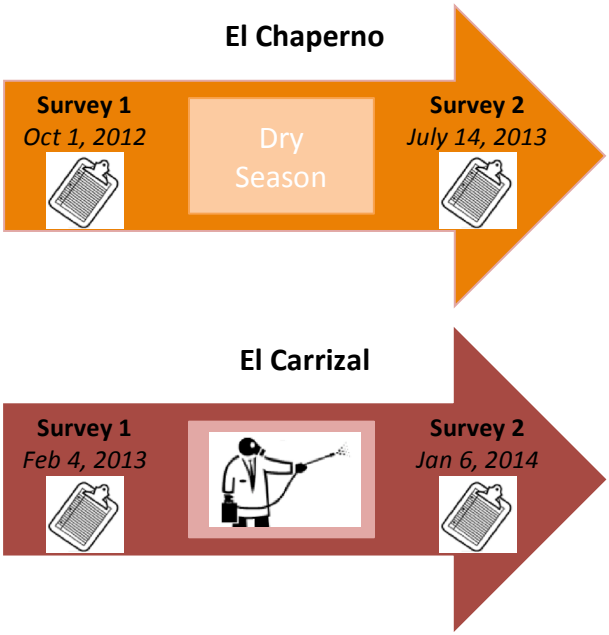

Figure 7. Collection and survey timeline in El Chaperno and El Carrizal.

Vector Collection. During the survey, a member of the local ministry of health searched thoroughly each household for the vector. Time of search ranged from 15 to 45 minutes. In the field, each *T. dimidiata* was aged, and adult were sexed. It is important to determine life stage and sex since this information will allow us to determine demographic distribution of the vector at each house. Insects were then preserved in 95% ethanol in numbered vials, at room temperature. DNA was extracted in Dr. Lori Stevens' lab at the University of Vermont. All samples will be amplified with *T. cruzi* primers, TCZ1 and TCZ2, to test for parasitic infection, and later compare with the results of NGS, as well as to use as a variable in our data analysis (Pizzarro *et al*. 2007). Subsequently, subsets of the samples will be genotyped by sequencing to obtain SNP markers for both vectors and parasite (if the insect is infected). Ninety-six vectors will be genotyped for each of the four sample periods. The selection criteria are as follows:

a. For domestic and peridomestic structures with three or more specimens, three specimens will be selected. Preferably we will select a male, a female, and the highest instar to make up the group of three insects.
b. For domestic and peridomestic structures that only have one or two specimens, all of the specimens will be used.
c. The remaining specimens (96 – total sum of specimens in steps 1 and 2) will be selected in equal numbers from the three domestic structures with the most *T. dimidiata.* Preference will be to select equal numbers of adults and lowest instars.

Geographic Data. The latitude and longitude of each house was recorded in the field using waypoint averaging on a Garmin eTrex® 20 GPS unit. In addition to the house locations, the location of streetlights, stores, churches, secondary roads (not visible on satellite imagery), were recorded using waypoint averaging. Having the position of the houses will allow us to estimate the distance among houses and directly compare it to the genetic distance among individuals. Satellite imagery will be used to digitize roads, rivers and highly vegetated regions. Digitized features will allow us to parameterize landscape data than can be then used identify boundaries, bridges, and reservoirs of the vector. Free GIS layers from the US government will be used for the climate and elevation data in addition to the data for El Carrizal and El Chaperno.

Analysis. To assess genetic diversity I will obtain the allele frequency from the genotype of each of the individuals and run F-statistics among houses that have 3 or more samples. I will also use the frequency value of the lesser allele to assess autocorrelation among my data within each town. Then, I will generate a Bayes tree using neutral alleles from the all individuals, and subsequently generate a patristic distance matrix. To assess connectivity of individuals, I will fit a spatial model (TBD) using information from a semivariogram, which will be generated using the patristic matrix. Then I will use simple kriging to assess the spatial connectivity of the samples' genetic

relatedness across town. This tool will allow me to look at the connectivity of insects from different sides of town, and assess the physical length at which reservoirs allow movement of insects within town. I will also use cross-semivariograms and cross-correlograms to associate genetic relatedness to factors such as infrastructure, house isolation, clutter, and presence of domestic animals across each town. These analyses will be done in conjunction with John Hanley, as he has developed most of the scripts to run spatial statistics in MatLab.

To address whether the dry season changes the structure of a population driving the introduction of new individuals, I will run STRUCTURE, which is program that allows the Bayes inference of population structure. I will quantify the increase in heterozygosity and population diversity, and run clustering analysis to determine if there are changes in the genetic clusters within the town once the dry season has ended. For the samples coming from the second collection, I will determine the proportion of insects within houses that are migrants versus insects that have been in the house over more than one generation. To do this I will use the software BIMR to try to identify sink populations within houses that have multiple instars to try to infer the proportion of insects within the house that dispersed during the previous generation (Faubet and Gaggiotti, 2008).

Finally, I will address the fumigation question, first by mapping the shifts of insect abundance and determining if there are newly formed clusters from recent migrations, or old clusters that were not successfully eradicated. I will be looking for edge effect, change in the population distribution, and clustering of insects within the center of the town. I will run STRUCTURE to assess if there is difference among early and late populations, and run a STRUCTURE cluster analysis to determine if the genetic structure of cluster has changed. Finally, I will assess spatial connectivity by testing genetic relatedness using geo-spatial analysis from patristic distances, and will test for edge effect using a simple kriging method in ArcGIS.

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Table 1. Academic credits for Graduate Degree

School	Dept.	Term	No.	Description	Credits*	Grade
OSU	STAT	Fall 2009	650	Analytical Interpretation of Data I	5	A-
OSU	ENTO	Fall 2009	631	Insect Physiology	5	B+
OSU	EEOB	Wtr 2010	700	Principles of Biogeography	5	A-
OSU	STAT	Wtr 2010	660	Analytical Interpretation of Data II	3	A
OSU	ENTO	Wtr 2010	790	Nature & Practice of Science	2	A
OSU	EEOB	Spr 2010	640	Genetic Basis of Evolution	5	A-
OSU	ENTO	Spr 2010	694	Insect Biodiversity	4	A-
OSU	STAT	Spr 2010	662	Environmental Stats	3	A-
OSU	ENTO	Fall 2010	641	Insect Ecology	4	A
OSU	ENTO	Spr 2011	650	Bio Control of Arthropods	4	A
UVM	MMG	Fall 2012	233	Genetics and Genomics	3	A-
UVM	NR	Fall 2012	343	Fndmtls of Geog Info Systems	3	A
UVM	PH	Fall 2012	302	Epidemiology	3	B+
UVM	BIO	Spr 2013	381	Special Topics: Phylogenetics	1	A
UVM	MMG	Spr 2013	232	Methods in Bioinformatics	3	A
UVM	NR	Fall 2013	242	Adv Geospatial Techniques	2	A
UVM	CE	Spr 2014	369	Applied Geostatistics	2	TBD
UVM	NR	Fall 2014	376	Teaching Methods	TBD	TBD

*Credits transferred from Ohio State to UVM were converted with a 2:3 ratio, adding up to 22 credits. Transfer credits will not be considered for current GPA, but count as research credits towards degree.

Table 2. Timeline

		Fall '12	Spr '13	Sum '13	Fall '13	Spr '14	Sum '14	Fall '14	Spr '15	Sum '15	Fall '15	Spr '16	Sum '16
Objective 1													
Task 1	Sample collection												
Task 2	DNA Extractions												
Task 3	RAD-seq												
Task 4	Pipeline Development												
Task 5	Parameterize SNP data												
Task 6	Paper writing												
Objective 2													
Task 1	DNA Extractions												
Task 2	Prepare GBS samples												
Task 3	GBS Pipeline												
Task 4	Map manipulation												
Task 5	Data analysis												
Task 6	Paper writing-spatial												
Task 7	Paper writing-temporal												
Degree Requirements													
Proposal Defense													
Teaching Requirement													
Comprehensive Exam													
Course Credits													
Research Credits													
Dissertation Writing and Defense													