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Research paper

Residual survival and local dispersal drive reinfestation by *Triatoma dimidiata* following insecticide application in Guatemala

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ABSTRACT

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* and transmitted by triatomine insect vectors. In Guatemala, insecticide spraying is an integral part of management of the main vector, *Triatoma dimidiata*. Spraying typically has low efficacy, which may be due to incomplete elimination from infested houses, within-village dispersal, or influx from other villages or sylvan environments. To evaluate how these mechanisms contribute to reinfestation, we conducted a time-course analysis of *T. dimidiata* infestation, abundance and household genetic structure in two nearby villages in Jutiapa, Guatemala; houses in the first village were surveyed, treated with insecticide if infested and then re-surveyed at eight and 22 months following spraying, while the second village served as an untreated control to quantify changes associated with seasonal dispersal. Insects were genotyped at 2–3000 SNP loci for kinship and population genetic analyses. Insecticide application reduced overall infestation and abundance, while the untreated village was stable over time. Nevertheless, within two years 35.5% of treated houses were reinfested and genetic diversity had largely recovered. Insects collected from reinfested houses post-spraying were most closely related to pre-spray collections from the same house, suggesting that infestations had not been fully eliminated. Immigration by unrelated insects was also detected within a year of spraying; when it occurred, dispersal was primarily local from neighboring houses. Similar dispersal patterns were observed following the annual dispersal season in the untreated village, with high-infestation houses serving as sources for neighboring homes. Our findings suggest that the efficacy of pyrethroid application is rapidly diminished by both within-house breeding by survivors and annual cycles of among-house movement. Given these patterns, we conclude that house structural improvements, an integral part of the Ecohealth approach that makes houses refractory to vector colonization and persistence, are critical for long-term reduction of *T. dimidiata* infestation.

1. Introduction

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*, which is transmitted by insects of the Triatominae subfamily (Reduviidae) in the Americas. It is widely distributed from southern USA to northern Argentina (Telleria and Tibayrenc, 2017). Among the 600 million people living in countries where the disease is endemic, 6.6 million are estimated to be infected as of 2015 (Global Burden of Disease Study 2015 Collaborators, 2016).

In Guatemala, the main Chagas vector is *Triatoma dimidiata* (Hemiptera, Reduviidae), a native species that can be found in domestic, peridomestic and sylvan environments (Dorn et al., 2007). Historically, control of Chagas disease transmission was attempted by applying residual insecticide inside houses and nearby out-buildings (Tabaru et al., 1998; Ramsey et al., 2003; Dumonteil et al., 2004; Hashimoto et al., 2006; Barbu et al., 2009; Cecere et al., 2013; Lucero et al., 2013; Yoshioka et al., 2015). This strategy was successful for some vector species, e.g. interruption of transmission by *Rhodnius prolixus,* an introduced species in Central America (Hashimoto and Schofield, 2012; WHO, 1997). However, differences in the ecology of *R.*

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prolixus and the native vector *T. dimidiata* suggested that similar intervention strategies may not always be equally effective (Dorn et al., 2003; Dumonteil et al., 2004; Hashimoto et al., 2006; Bustamante et al., 2009; Weeks et al., 2013). Insecticide effectiveness can decline rapidly in some locations (3–6 months), and treatment may not reduce peridomestic and sylvan populations that can rapidly reinfest houses (Barbu et al., 2014; Bustamante et al., 2009; Tabaru et al., 1998). Historically, national health institutions prioritized use of pyrethroid insecticides to control vector populations (Dumonteil et al., 2004; Barbu et al., 2011; WHO, 2015); however, in response to the lack of long-term effectiveness of insecticides against *T. dimidiata*, complementary Ecohealth strategies for improving houses have been developed and are gaining acceptance in some but not all localities (Zeledón and Rojas, 2006; Monroy et al., 2009; 2012; Lucero et al., 2013; Gürtler and Yadon, 2015; De Urioste-Stone et al., 2015; Yoshioka et al., 2015; Zamora et al., 2015).

To optimize vector control strategies, we need to understand the mechanism(s) of the recovery of triatomine populations following conventional insecticide application. One potentially important contributor to reinfestation may be residual survival. Eggs can survive insecticide, and among all of the *T. dimidiata* life stages, third to fifth instar nymphs are most likely to avoid a chemical treatment given their ability to fit deeper into crevices, survive weeks without a blood meal, and reduce their feeding to a single blood meal per stage (Zeledón et al., 1970a). Adaptive behavior in domestic ecotopes, such as hiding deep in wall crevices, floors or roofs, increases the possibility of surviving insecticide treatments (Zeledón et al., 1970a; Bustamante et al., 2009; Monroy et al., 2009). Furthermore, given that a fertilized female adult can lay an average of 177 fertile eggs in her life span (Zeledón et al., 1970b), even a small number of individuals that survive an insecticide treatment could rapidly re-populate a house.

A high propensity for movement may also facilitate reinfestation. Peridomestic environments are not always targeted for insecticide treatment, and studies conducted in the regions of Yucatán Peninsula by Dumonteil et al. (2004) and in Petén, Guatemala by Monroy et al. (2003), recorded rapid re-colonization from peridomestic ecotopes associated with an annual dispersal peak at the end of the dry season, between the months of March through May. Houses that remain infested may also serve as "hotspots" from which insects recolonize other houses either in close proximity or from a greater distance (Cecere et al., 2004; Stevens et al., 2015). Migration may also occur from sylvan environments or neighboring villages, possibly via human-mediated transport (Dorn et al., 2003; Monroy et al., 2003; Ramirez-Sierra et al., 2010; Barbu et al., 2011, 2014; Gourbière et al., 2012; Bustamante et al., 2014; Stevens et al., 2015). Thus, understanding the timing, distance, and sources of movement in *T. dimidiata*, both with and without insecticide application, is critical for determining the appropriate type and scale of intervention required for effective long-term management of transmission risk.

In this study, we used fine-scale genetic analysis to evaluate the relative contributions of within-house persistence, sylvan influx and local dispersal to household reinfestation by *T. dimidiata* following insecticide treatment in the state of Jutiapa, Guatemala. We assessed infestation rates and patterns of spatial genetic structure at multiple time points in two villages, one of which was treated with insecticide once during the survey period. We combined survey data with vector kinship and population-genetic analysis using 2–3000 genome-wide single nucleotide polymorphic markers (SNPs) obtained from a genotyping-by-sequencing (GBS) pipeline to evaluate: (1) the effectiveness of insecticide application in reducing abundance and genetic diversity over time, (2) whether population recovery is due to survival of insecticide application and/or reinfestation by dispersers, and (3) the spatial scale of seasonal dispersal.

2. Methods

2.1. Study sites and entomological surveys

To examine patterns of dispersal and spray recovery, two villages in the Department of Jutiapa, Guatemala were surveyed for vector infestation and abundance over time; in one village surveys encompassed an insecticide application event (El Carrizal), while in the second, surveys spanned the annual dispersal season in the absence of insecticide (El Chaperno). Both El Carrizal (14.3766, −89.9863, 700 masl) and El Chaperno (14.3491, −89.9468, 700 masl) are rural communities in the dry highlands, divided by a mountain range crossing North to South (Fig. 1). The National Institute for Seismology, Volcanology, Meteorology and Hydrology in Guatemala reports an average annual precipitation for Jutiapa of 1633 mm concentrated in the months of May through October, peaking in the month of September (INSIVUMEH meteorological database, 2012; http://www.insivumeh.gob.gt/). The Euclidean distance between village centers is 5.26 km, with an effective road distance of 11.6 km. In both villages, most houses are built with adobe and other local materials, with fewer than 10% built from cement blocks. The vector control program in both villages was based solely on insecticide applications of deltamethrin, a third generation pyrethroid; the Ministry of Health reported the most recent insecticide campaigns were three years prior to the study period for El Carrizal and four years prior for El Chaperno.

In El Carrizal, a pre-spray survey was conducted in February 2013 and all positively-infested houses (containing one or more live *T. dimidiata)* were treated with deltamethrin by Department of Health staff between the months of March and May 2013, according to the Guatemalan Department of Health Vector Control Protocol (Jutiapa, Guatemala) (Table 1). Briefly, 1–2 spray packs of a 5% solution of deltamethrin wettable powder (100 g/pack), were applied with an X-pert compression sprayer (H.D. Hudson, Chicago, IL) in a single application at ~25 mg/m^2 on indoor surfaces (walls, floors and bed) as well as peri-domestic structures such as chicken coops, corrals, and ovens. Eight months after spray completion (Jan. 2014), the first of two post-spray surveys was coordinated to collect specimens and assess differences in abundance after the insecticide treatment. Houses were not treated at this time. Twenty-two months after insecticide application (April 2015), a second post-spray survey was conducted (Table 1). In El Chaperno, houses were surveyed in the first week of Oct. 2012, eight months prior to a scheduled insecticide treatment program for the village, at the end of the rainy season and prior to the putative annual dispersal season. Following the first survey, a visit offering education and tips for Chagas prevention was organized by the Ministry of Health personnel. Eight months after the first collection, a post-dispersal season survey was conducted amid the following rainy season in July 2013 (Table 1). In both villages, once the final survey was completed, the Ministry of Health treated all houses identified as infested from any of the surveys with deltamethrin. All survey and insecticide protocols were approved by the Comité de Ética en Salud del Gobierno de Guatemala resolución 14–11. No refusals of insecticide application were reported by the Ministry of Health.

Surveys to search for *T. dimidiata* were conducted as a collaboration with Laboratorio de Entomoligía Aplicada y Parasitología (LENAP) at San Carlos University of Guatemala and the National Vector Control Program implemented by Guatemala's Ministry of Health (Jutiapa, Guatemala). Ministry of Health inspectors searched for *T. dimidiata* inside houses on walls, floors, in clutter, animal nests and personal belongings (Monroy et al., 1998a). All houses from both villages were geo-referenced (waypoint error = ± 6 m) during the initial surveys using Garmin eTrex® GPSs, set to WGS 84 map datum. The *T. dimidiata* specimens located during each survey were collected live, transferred to vials containing 95% ethanol, stored at room temperature at LENAP and transported within a week to the University of Vermont where they were stored at −20 °C until DNA was extracted for sequencing.

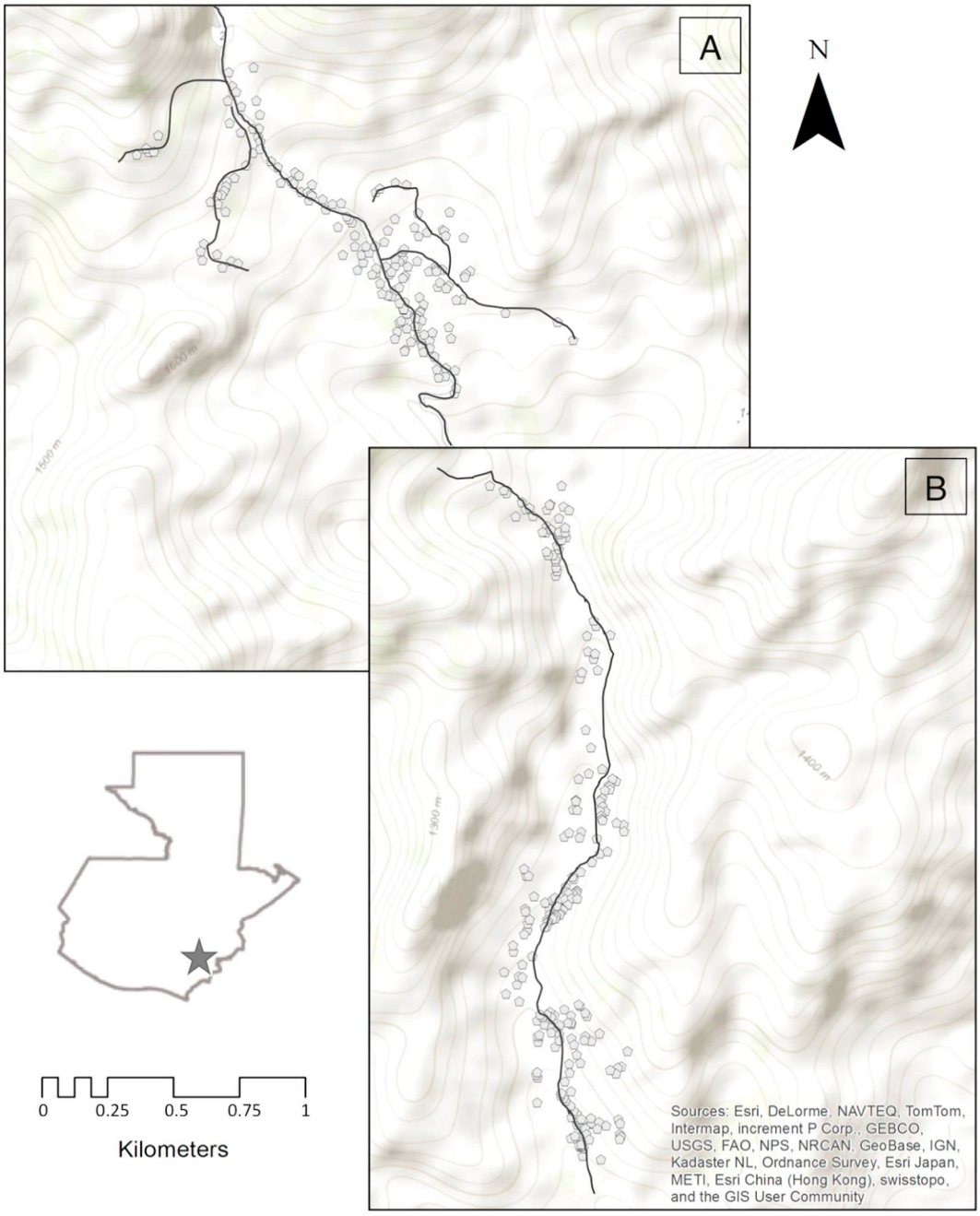

Fig. 1. Map of individual house locations in El Carrizal (A) and El Chaperno (B), Jutiapa, Guatemala. Insert shows the location of the Department of Jutiapa in Guatemala. Source by ArcGIS 2009 (ESRI Inc., Redlands, CA).

2.2. DNA extraction and GBS sequencing

DNA was extracted from a total of 834 *T. dimidiata* specimens, 382 from El Carrizal and 452 from El Chaperno. Numbers and life stages of insects collected and sequenced from each house are provided in Tables S1 and S2. DNA was extracted from individuals stage III-adult, as well as stage I-II nymphs when older individuals were not sampled from a house. Because restriction-site associated genotyping methods are sensitive to DNA quality (Orantes et al. 2018), specimens were excluded from sequencing due to low yield or quality, as indicated by molecular weight assessed on a 1.5% agarose gel. Houses with larger numbers of insects tended to include a higher proportion of nymphs, which are less likely to disperse (Monroy et al., 2003) and thus could systematically under-estimate dispersal from houses with larger sample collections. To avoid such bias, high-infestation houses (over three insects collected) were sub-sampled (range = 3–21 insects/house), and adults were prioritized if collected, followed by 4th–5th stage nymphs and lastly 1st-3rd instar stage nymphs. DNA was extracted from either three posterior segments of the insect abdomen and one surface-sterilized leg (including attached muscle) or the thorax. We also extracted the DNA from 12 surface-sterilized leg specimens collected from across the range of *T. dimidiata* to build a DNA reference catalog of 5177 tags to identify vector sequences separate from contaminating microbes and blood meals as described in Justi et al. (2018). The DNA from the specimens was extracted by flash freezing the tissue in liquid nitrogen, homogenizing it with a bead-based homogenizer and using a modified Qiagen DNeasy™ tissue extraction protocol. Modifications included a 12-hour Proteinase K digestion at 56 °C, followed by an RNAse digestion at 37 °C for 30 min using 1.0uL of 4 mg/mL RNAse. DNA was quantified using a Qubit spectrophotometer high-sensitivity protocol,

Table 1
Sampling regime for the villages of El Carrizal and El Chaperno, Jutiapa, Guatemala.

Location	Survey date	Houses surveyed	% of town surveyed	Sampling period	Insecticide regimen
El Carrizal	Feb 4, 2013	129	82	Pre-spray	Sprayed 100% positive houses (March–May 2013)
El Carrizal	Jan 6, 2014	128	81	8-months post-spray	No treatment
El Carrizal	April 29, 2015	146	92	22-months post-spray	
El Chaperno	Oct 1, 2012	183	89	Pre-dispersal season	No treatment
El Chaperno	July 16, 2013	193	94	Post-dispersal season	

and quality of the molecular weight was determined by electrophoresis on a 1.5% agarose gel stained with Sigma-Aldrich Nancy-520™. Only specimens with a minimum yield of 900 ng of DNA and molecular weight above 600 bp were considered suitable for sequencing; because *T. dimidiata* are blood-feeding and the abdomen can contain significant amounts of degraded blood meals, DNA quality was variable. Of the original 834 extracted specimens, a total of 433 were sequenced, which included 115 pre-spray, 66 early post-spray and 62 final post-spray samples from El Carrizal, and 91 pre-dispersal season and 99 post-dispersal season samples from El Chaperno. Of these, 91 were females, 89 were males, and the remaining 253 were nymphs (Table S1).

A GBS library was prepared using the restriction enzyme *PstI* (6-base cutter: 5′ — CTGCA↓G — 3′, 3′ — G↓ACGTC — 5′) at the Cornell Genomic Diversity Facility (Ithaca, NY) following the Genotyping-by-Sequencing (GBS) protocol of Elshire et al. (2011). Each specimen was barcoded, and multiplexed in a 48-plex format on an Illumina HiSeq Analyzer. The raw sequencing reads were 93 bp in length, including the inline 5-bp barcode and 6-base*PstI* recognition sequences. We used FastX-trimmer in the FastX-toolkit to remove the barcodes and recognition sites (Gordon and Hannon, 2010). The trimmed 82-bp long sequences were filtered using FastQ-quality-filter to remove sequences with any base having a confidence score below 10.

The 433 sequenced specimens were mapped to the reference catalog with Bowtie (Langmead, 2010). Genotypes were called using the Stacks *ref_map* pipeline independently for El Carrizal and El Chaperno to include only loci polymorphic for each village (Catchen et al., 2013). We set the parameters for the reference mapping to a depth of coverage of 3×, allowing three mismatches among tags from the same individual and three mismatches between individuals. We excluded any SNP that was not represented in at least 50% of the samples and those that violated Mendelian genetics in any sample (i.e., > 2 alternative bases at the SNP). After filtering, the pipeline yielded a total of 2780 informative SNPs for El Carrizal and 2263 for El Chaperno.

2.3. Infestation index and vector abundance

Changes in the proportion of surveyed houses found to be infested (the infestation index, WHO, 2002) across time points were evaluated with Chi-Squared tests. To test for temporal reduction in abundance within infested houses due to insecticide application, differences in the abundance and the proportion of nymphs in the subset of houses identified as positive in the pre-spray survey were evaluated with mixed-model GLMs using the JMP statistics package (ver. 9, SAS Institute Inc., Cary, NC). Survey period was included as a fixed factor, with house ID as a random factor. Similar analysis was conducted for El Chaperno; in this case, because dispersal may lead to an increase in infested houses, all houses with infestations at one or both survey periods were included in the analysis.

To test for spatial clustering of *T. dimidiata* infestation, the locations of all houses were mapped using individual GIS data layers with a UTM zone 16 NAD27 map projection in ArcGIS 10.1 (ESRI Inc., Redlands, CA). For each survey, houses were classified as infested or uninfested and the spatial distribution of infested houses was tested for spatial clustering using the Average Nearest Neighbor function with inverse distance weighting from the spatial statistics toolbox (ArcGIS, ver. 10.0, ESRI Inc.).

2.4. Household T. dimidiata genetic structure

For each sampling point, we estimated pairwise kinship values with the Identity by Descent (IBD) kinship coefficient using Maximum Likelihood Estimation (MLE) with the *snpgdsIBDMLE* function in the *SNPrelate* R package (Zheng et al., 2012). The kinship value k is expected to be 0.5 for parent-offspring or full-sib relationships, with a decay toward zero for more extended relatives (e.g., 0.25 for half-sibs, 0.125 for first cousins, etc.) and zero for unrelated individuals. For each village, we calculated density curves of within- and among-house kinship for each survey and tested for differences in kinship patterns within versus among houses within each sampling period, as well as differences in within-house kinship distributions over time, using non-parametric, pairwise Kolmogorov-Smirnov Tests (K-S test, alpha = 0.05) from the R package *rgr* (Garrett, 2013).

To identify the sources of post-spray infestations, we tested whether insects from reinfested houses were more likely to have been derived from pre-spray infestations from either a) the same house, or b) a nearby house, than from a random reinfestation source across the village. Because potential source houses varied in insect abundance, and therefore potential dispersers, prior to insecticide application, we used a randomization test to generate expected colonization patterns under random dispersal given observed variation in initial abundance. Pairwise kinship values were calculated across the entire set of sampling periods; to calculate observed reinfestation patterns, for each *post-spray* insect collected from a house reinfestation (i.e., that house had also been infested in the pre-spray survey), the *pre-spray* sample to which it was most closely related was identified. If the nearest relative was collected from the same house, it was classified as a residual infestation; if not collected from the same house, the Euclidean distance between the two houses was calculated as an estimate of infestation dispersal distance. The observed proportion of residual infestations and the mean distance to the source house for non-residual colonizations were compared to the distribution of expected values calculated from randomized datasets, in which each post-spray sample was assigned a randomly selected pre-spray sample as its nearest relative. Calculations from randomized assignments were replicated 1000 times and the observed results compared to the 95%, 99% and 99.9% most extreme values as probability cutoffs. The randomization test was conducted with a custom-designed python script, available in Supplementary information.

2.5. Population genetic structure

To identify changes in overall population genetic diversity and structure in each village over time due to insecticide application or dispersal activity, the population fixation index (F_{ST}), nucleotide diversity (Pi), inbreeding coefficient (F_{IS}), observed (Het_{ob}) and expected (Het_{ex}) heterozygosity were calculated for each village and sampling period using the *Populations* function from the *Stacks* software (Catchen et al., 2013).

To identify genetic clusters and cluster assignment of the genotyped individuals from each village, we performed k-means clustering analysis and classified the individuals by a discriminant analysis of principal components (DAPC) using the *Adegenet* package for R (Jombart and Ahmed, 2011). Insects with < 5% of the average number of SNPs

were excluded from the analysis to prevent biases due to missing data; this amounted to 13 insects from El Carrizal and 11 from El Chaperno. We identified the number of genetic clusters for each village using the k-means cluster algorithm (from the *find.clusters* function in *Adegenet*) based on the value of k that minimized the Bayesian Information Criterion (BIC) value. For El Carrizal, we set the maximum number of potential principal components (PCs) to 240, retaining a total of 5 clusters based on the cumulative variance explained by the BIC values. One cluster consisted of a single individual (CHJ484), thus we re-ran the analysis excluding this sample. The final DAPC was best explained with 4 clusters and CHJ 484 as an outlier. For El Chaperno, we set the maximum number of potential PCs at 180, retaining a total of 2 clusters, based on the cumulative variance explained by the BIC. The cluster identity for each insect was used to project the cluster distributions for each village and each survey using ArcGIS 10.1 (ESRI Inc., Redlands, CA) (UTM zone 16, datum = WGS84). Changes in cluster proportions over time were tested with an RxC G-test of heterogeneity, with post-hoc pairwise comparisons when significant overall effects were detected.

To test for spatial structuring in the distribution of the DAPC genetic clusters, we created a matrix of pairwise Jaccard indices, which measures the overlap in sample cluster membership between two houses, and combined it with the pairwise Euclidean distances between houses using the *vegdist* function from the R *Vegan* package (Oksanen et al., 2007). To project the coordinates to meters, we converted the degree coordinates to UTM projections (zone 16, datum = WGS84) using the *coordinates*, *proj4string*, and *spTransform* functions from the linked packages *rgdal* and *sp* in R (Bivand et al., 2014; Pebesma and Bivand, 2005). We tested for spatial autocorrelation with Moran's I correlograms for each village and survey using the *correlog* function from the *ncf*R package (Hijmans and van Etten, 2014). The spatial increments (bin size) were selected as 25 m for El Carrizal and 20 m for El Chaperno using the Freedman-Diaconis rule for bin width using a histogram from the longitude distance matrix generated by the *hist* function in the R *Vegan* package (Oksanen et al., 2007). To assess clustering significance at each bin, we ran a permutations test for 100 repetitions (alpha = 0.05) using the *resamp* parameter from the *correlog* function. We determined the appropriate window to observe spatial autocorrelation within each village by using the Ripley's K function to establish the maximum spatial range among houses using the Spatial Statistics toolbox from ArcGIS 10.1 (ESRI Inc., Redlands, CA). The window was set to 580 m for El Carrizal and 480 m for El Chaperno. The 95% confidence interval (CI) was calculated from the Moran's I response vector using the *boot.one* function from the *boot* R package at 10,000 bootstraps and calculating the Bias Corrected and Accelerated Confidence Interval (BCa) for non-parametric functions with the *boot.ci* function from the *boot* R package (Angelo and Ripley, 2016; Davison and Hinkley, 1997).

To test whether insecticide application resulted in adaptive allelic changes at any of the identified SNPS, we tested for outliers in F_{ST} calculated across sampling timepoints in the treated village of El Carrizal using Bayescan 2.1 (Foll and Gaggiotti, 2008), with the untreated village El Chaperno evaluated as a control. An FDR-corrected *p*-value of 0.05 was used to identify significant outlier loci.

3. Results

3.1. Infestation index and vector abundance

The number of *T. dimidiata* collected per house was highly variable, ranging from no insects to a maximum of 81. The infestation index in El Carrizal declined significantly from 24.0% (31 of 129 houses) in the pre-spray survey to 12.4% in the 8-month post-spray survey (16 houses, $X_1^2 = 5.72$, $p < .016$), and remained significantly lower at the 22-month post-spray survey (17 houses, 13.2%; $X_1^2 = 7.29$, $p < .006$). In total, 25 houses were found to contain *T. dimidiata* at one or both of the post-spray surveys, including 11 (35.5%) of the houses initially treated for infestation and 14 new infestations (Table S2). For the subset of houses infested prior to insecticide application, per-house *T. dimidiata* abundance was significantly lower post-spray than pre-spray at both eight and 22 months following insecticide application, dropping from 7.73 ± 2.04 insects per house to 1.42 ± 0.79 after eight months and 2.58 ± 0.99 at 22 months after insecticide application (mixed-model GLM, main effect of time point, $F_{2,50} = 7.36$, $p < .01$; pre-spray vs. 8-months post-spray, $p < .01$, pre-spray vs. 22-months post-spray, $p < .05$). Nymphs made up an average of 65–78% of the insects collected per house, with no significant difference in demographic composition across surveys ($F_{2,61} = 0.78$, $p = .46$). No spatial clustering of infested houses was found in the pre-spray survey (Fig. 2A). However, eight months after the insecticide application, significant clustering was observed among infested houses, mostly driven by the presence of nymphs ($R = 0.831$, $z = -4.29$, $p = .019$) (Fig. 2B). The 22-month post-spray survey also showed significant clustering among infested houses driven by the presence of nymphs ($R = 0.644$, $z = -10.13$, $p < .001$) and males ($R = 0.813$, $z = -6.91$, $p < .018$) in the houses (Fig. 2C).

In the absence of insecticide application, neither infestation index nor per-house abundance in the village of El Chaperno changed following the putative dispersal season. There were 30 infested houses in 2012, representing 21.6% of the surveyed houses in the village, and 32 in 2013 (21.3%), of which 17 houses were recurrently infested in both surveys. No significant spatial clustering of infested houses was detected at either sampling time point (Fig. 3).

3.2. Household genetic structure

In both villages, patterns of genetic relatedness were strongly structured at the house level. Genetic kinship was significantly higher for insects collected from the same house than those collected from different houses (Kolmogorov-Smirnov two-sample tests, all $p < .0001$; Figs. 4,6). In El Carrizal, the distribution of within-house kinship values shifted significantly from the pre-spray to the 8-month post-spray survey (Kolmogorov-Smirnov test, $D = 0.121$, $p < .005$), from a unimodal distribution consistent with an extended family (median k = 0.104) in the pre-spray survey (Fig. 4A), to a distribution of similar median (k = 0.091) but multimodal in pattern, with density peaks for unrelated (k = 0.0), distantly related (k = 0.08) and more closely-related individuals (k = 0.18) (Fig. 4B). The 22-month post-spray distribution was again unimodal (median k = 0.082) and did not differ significantly from either the pre-spray or the 8-month post-spray distribution (Fig. 4C). When post-spray samples collected from houses that had also been infested prior to insecticide application were compared to the entire set of pre-spray samples, 47.2% were most closely related to a sample from the same house, significantly more likely than that expected by chance (randomization test, $p < .001$, threshold cutoff = 19.4%). When samples were most closely related to a pre-spray insect from a different house, the mean distance between houses was significantly shorter than that expected if dispersal had occurred at random (randomization test, $p < .001$; Fig. 5).

Kinship distributions within houses also shifted significantly in El Chaperno following the March–May dispersal season ($D = 0.209$, $p = .0017$), from a multi-modal distribution in the pre-dispersal survey (median k = 0.093) with distant (k = 0.04), close (k = 0.17), and parent/sibling (k = 0.5) kinship peaks, to a right-skewed distribution in the post-dispersal survey (median k = 0.061) predominantly driven by a peak of distant kinship (k = 0.04) but lacking the higher-relatedness modes from prior to the dispersal season (Fig. 6).

3.3. Population genetic structure

Although when taken together, survey period had a marginally non-significant effect on per-locus allelic diversity (Pi) in El Carrizal

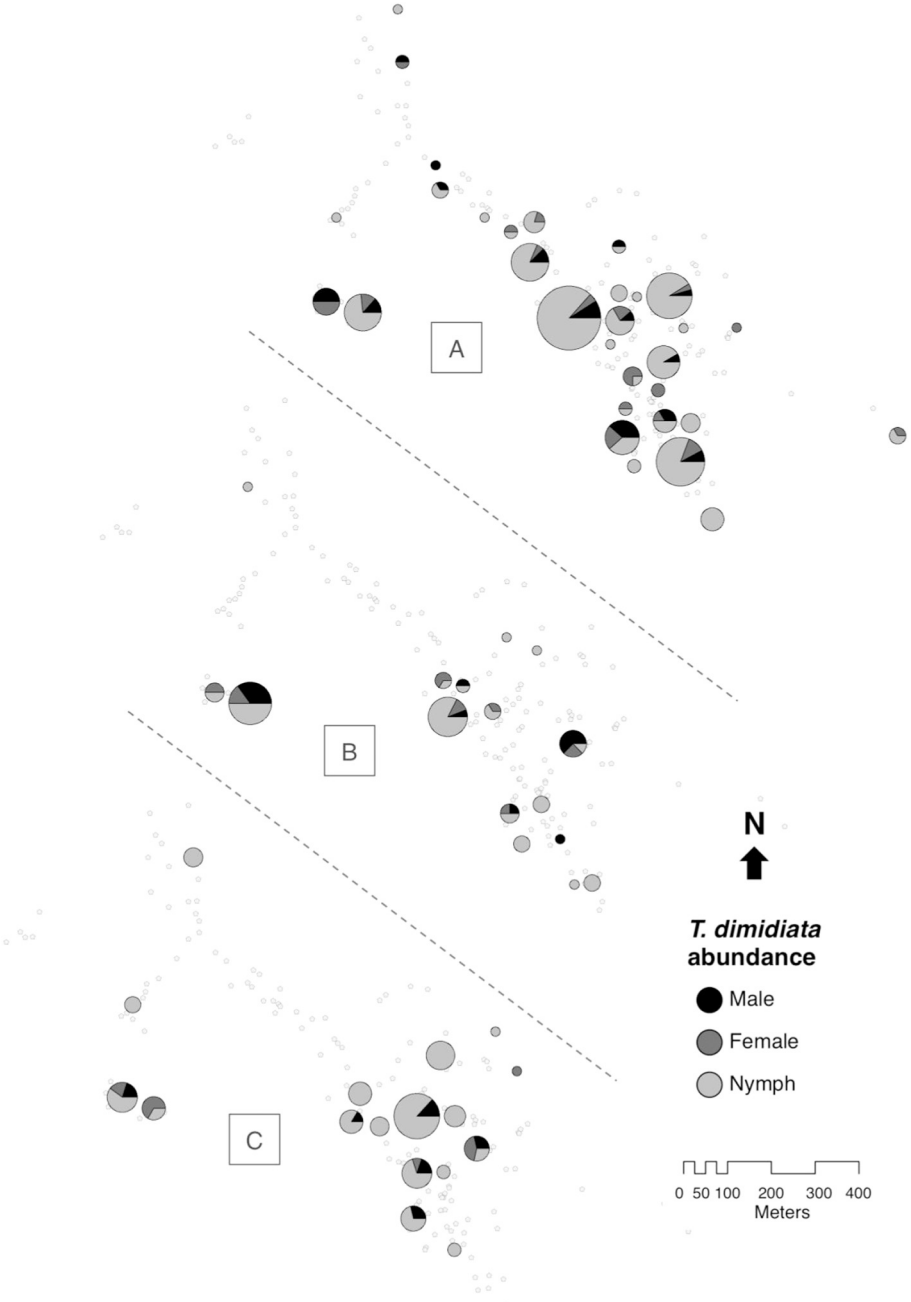

Fig. 2. Spatial distribution of *T. dimidiata* infestation in El Carrizal. Males (black), females (dark grey) and nymphs (light grey) were quantified across (A) the pre-spray survey in February 2013 (males, $n = 28$; females, $n = 30$; nymphs, $n = 163$), (B) an early post-spray in January 2014 (males, n = 16; females, $n = 13$; nymphs, $n = 46$), and (C) a final survey in April 2015 (males, $n = 12$; females, $n = 11$; nymphs, $n = 83$). Symbols are sized in proportion to the total vector density observed.

(ANOVA, $F_{2,3489} = 2.90$, $p = .055$); pairwise comparisons revealed a significant decrease from the pre-spray survey to the 8-month post-spray survey (Tukeys HSD test, $p < .05$), that recovered to an intermediate value by the 22-month post-spray survey which did not differ significantly from either the pre-spray or 8-month post-spray (Table 2). Population samples for both villages showed very low, though statistically significant, genetic differentiation over time ($F_{ST} \sim 0.005$), with the exception of the 8-month and 22-month post-spray periods in El Carrizal, for which differentiation nearly doubled ($F_{ST} = 0.0085$; Tables 2,3). The population inbreeding coefficient (F_{IS}) decreased from 0.29 to 0.15 from the pre-spray to the 8-month post-spray survey, increasing to 0.20 by the 22-month post-spray survey (Table 2). F_{IS} in El Chaperno was similar to the pre-spray value for El Carrizal both prior to and following the dispersal season (Table 3). Insecticide treatment did not result in detectable shifts in allele frequencies of any of the SNP markers that would be indicative of insecticide-mediated selection. We tested for F_{ST} outliers in each village across sampling time points with Bayescan; with an FDR cutoff of 0.05, no outlier loci were detected in either village (Supplementary Fig. 7).

The specimens from all three surveys ($n = 245$) clustered into four genetically distinct clusters, all of which were present in the pre-spray collection (Fig. 8A). Cluster representation within the population changed significantly across sampling periods (G-test of heterogeneity, $G_6 = 21.5$, $p < .01$). This overall effect was driven by a shift between the pre-spray and 8-month post-spray samples ($G_6 = 15.8$, $p < .02$). Cluster 1 dropped from 18% of the population in the pre-spray survey to only 3% in the 8-month post-spray survey, and cluster 4, the rarest initially, was not detected in the 8-month post-spray population

Fig. 3. Spatial distribution of *T. dimidiata* infestation in El Chaperno. Males (black), females (dark grey) and nymphs (light grey) were quantified during (A) prior to the dispersal season in Oct 2012 (males, $n = 50$; females, $n = 29$; nymphs, $n = 199$), and (B) after the dispersal season in July 2013(males, $n = 36$; females, $n = 24$; nymphs, $n = 137$). Symbols are sized in proportion to the total vector density.

(Fig. 8B). All four were found in the final post-spray sampling (Fig. 8C), and the composition of the population had returned to proportions not different from the pre-spray survey ($G_6 = 4.4$, $p = .62$). The spatial distribution of the groups remained identical across time points: groups one and two were distributed in the southwest main corridor of the village, while three and four occurred in the northwest and northeast outskirts of the village, respectively (Fig. 8). Consistent with their spatial segregation, houses were positively spatially autocorrelated in cluster membership in both the pre-spray and 22-month post-spray surveys up to a range of 362 m (Fig. 9A, C). No consistent pattern of spatial autocorrelation was detected in the 8-month post-spray survey, although the small number of infested houses reduced the power of the test for most distance classes ($n = 1$–16 comparisons per distance class; Fig. 9B).

Movement during the dispersal season alone did not alter population-genetic parameters in El Chaperno (Table 3). The overall *T. dimidiata* population, including both pre- and post-dispersal season samples, was separated into two genetically distinct clusters, each containing roughly similar proportions of individuals at each sampling point (prior to dispersal: 0.39:0.61; following dispersal: 0.53:0.47) (Fig. 10). Prior to dispersal, spatial autocorrelation of house cluster membership was limited to short and relatively long distances (38 m, 370–390 m; Fig. 11A); following the dispersal season, however, genetic identity was positively autocorrelated up to a range of 150 m (Fig. 11B).

4. Discussion

Targeted insecticide treatment is an economically attractive method for controlling Chagas disease vectors in Central America, yet its effectiveness can be short-lived. The results of this study suggest two main reasons for population recovery of the vector *T. dimidiata* in Guatemalan villages. First, targeted insecticide application is ineffective

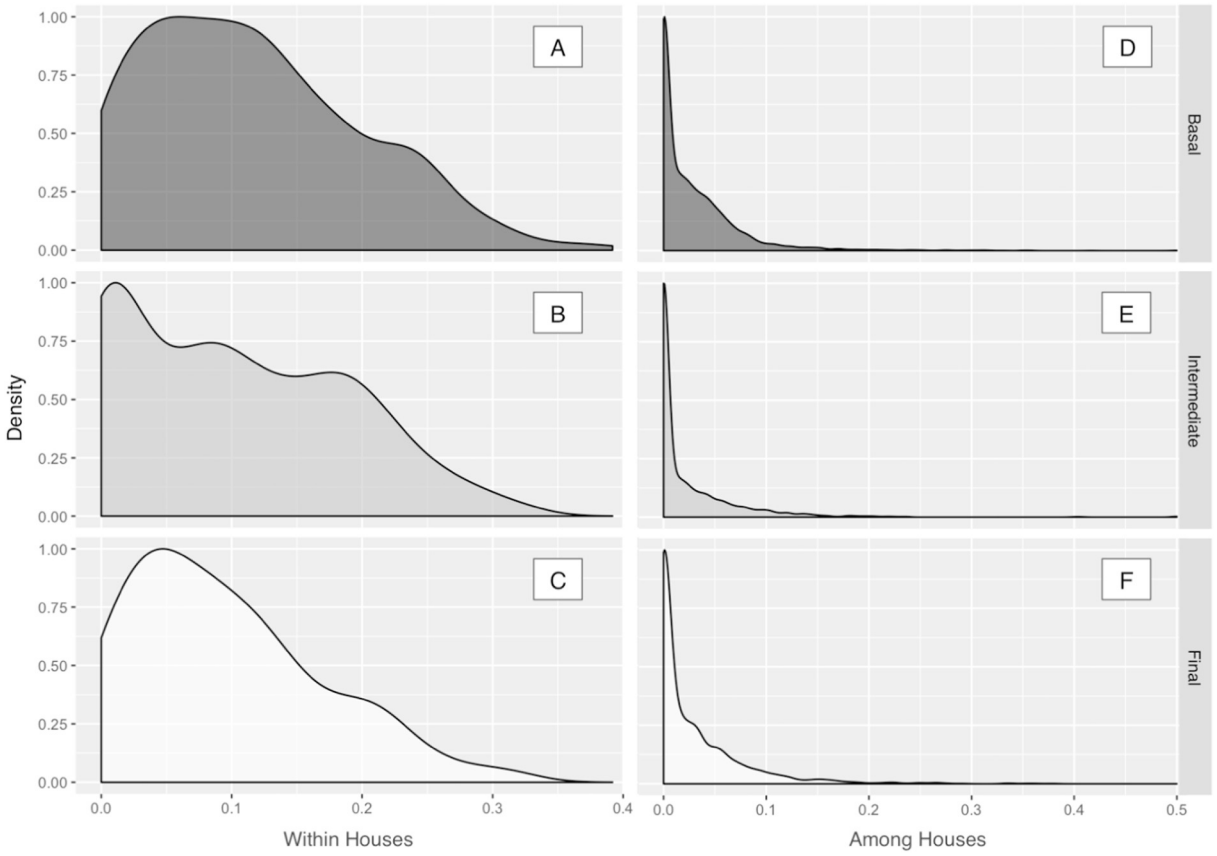

Fig. 4. Kernel density distributions of genetic kinship among *T. dimidiata* individuals within and among houses in El Carrizal, Jutiapa, Guatemala. Density curves are shown for within-house and among-house pairwise relatedness, respectively, for the pre-spray (A & D), early post-spray (B & E) and final post-spray (C & F) surveys. Calculations of pairwise-kinship were done for each timepoint separately using a Maximum Likelihood Estimation (MLE) of Identity by Descent (IBD) between individual genotypes. For ease of comparison, y-axis density values are standardized to the height of the highest peak of the curve (= 1.00).

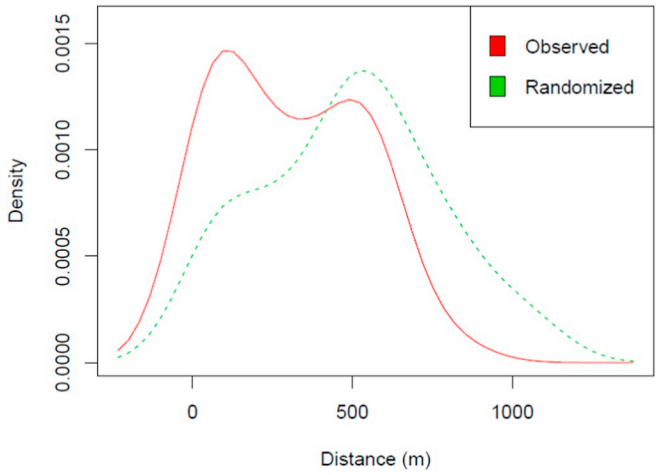

Fig. 5. Comparison of observed and randomized distributions of nearest-relative distance following insecticide application. The randomized curve represents the output of a single randomly-selected run (out of 999) from the random-assignment simulation. The mean distance from the observed distribution is significantly smaller than that expected from random dispersal ($p < .001$).

at completely eliminating infestations from individual households, resulting in endemic recovery as survivors reproduce and repopulate available domestic niches. Second, houses with surviving insects, whether due to initial non-detection (Monroy et al., 1998a) or survival of insecticide treatment, can serve as a local reservoir for reinfestation of nearby houses. Sylvan influx appears to be a less important mechanism of reinfestation than within-village processes, which is consistent with the high level of deforestation in this region of Guatemala.

Although insecticide treatment reduced the size and genetic diversity of the vector population, 31% of the treated houses were reinfested by the end of the study; the relatively rapid genetic recovery suggests that insecticide application was not effective at reducing the overall gene pool or preventing admixture among genotypes within the village. These results are similar to those of Dorn et al. (2003), Ramirez-Sierra et al. (2010) and Stevens et al. (2015), which have shown systematically high genetic diversity in *T. dimidiata* despite periodic insecticide application campaigns both within villages and across the region since 2000. Notably, both genetic diversity and the allelic composition of the population after 18 months closely resembled the pre-spray population (Table 2, Fig. 8), with no evidence of a severe genetic bottleneck, selection for particular genotypes (Fig. 7), or influx of novel genotypes from sylvan environments or other localities in the region. It is important to note that this result does not preclude possible evolutionary changes at loci influencing insecticide resistance; more comprehensive or gene-enriched SNP genotyping would be required to identify specific genomic regions responsive to insecticide-mediated selection.

Identifying the source of reinfesting insects is an important element of designing improved control strategies. Previous studies have utilized a variety of morphological and molecular methods to distinguish immigrants from persistent natal populations, with varying degrees of power and specificity (e.g., morphometry, Hernandez et al., 2013; mtDNA sequence variation, Quisberth et al., 2011; microsatellites, Stevens et al., 2015). The SNP markers derived from GBS sequencing

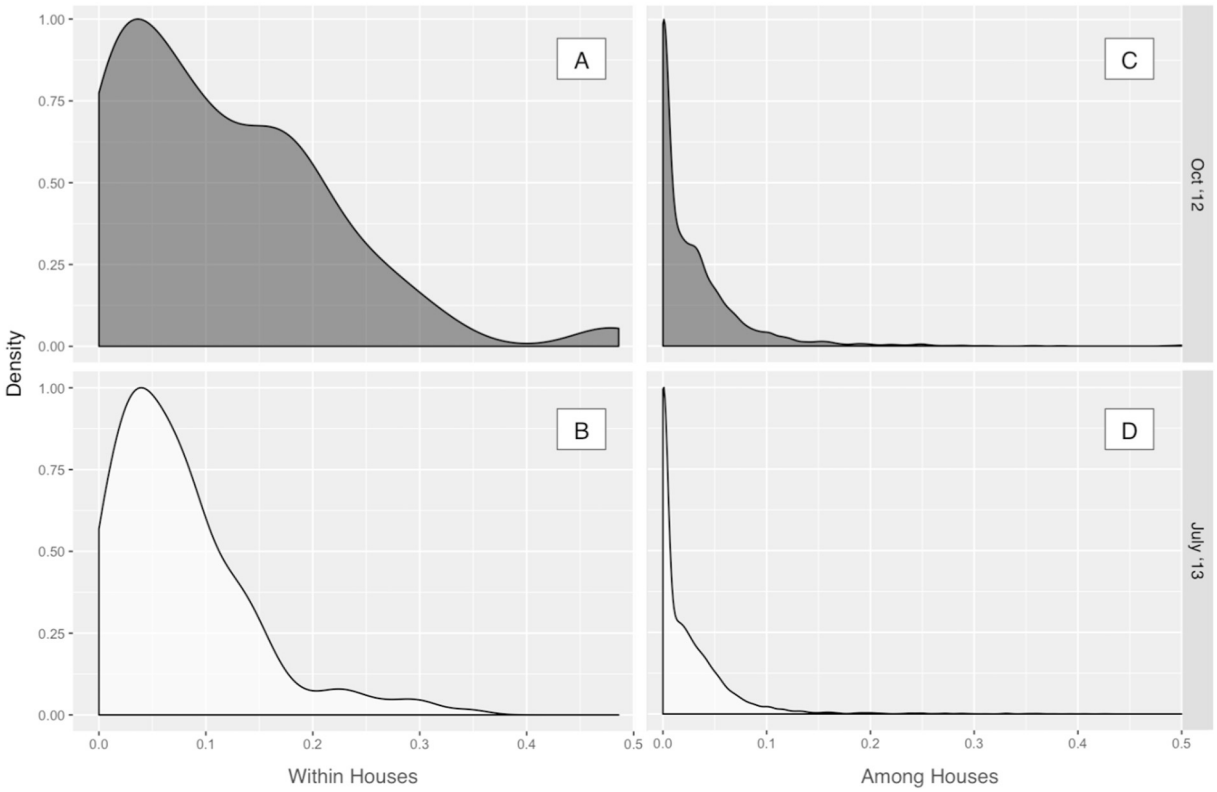

Fig. 6. Kernel density distributions of genetic kinship among *T. dimidiata* individuals within and among houses in El Chaperno, Jutiapa, Guatemala. Density curves are shown for within- and among-house pairwise relatedness, respectively, for the pre-dispersal season (A & C) and post-dispersal season (B & D) collections. Calculations of pairwise-kinship were done for each collection period separately using a Maximum Likelihood Estimation (MLE) of Identity by Descent (IBD) between individual genotypes. For ease of comparison, y-axis density values are standardized to the height of the highest peak of the curve (= 1.00).

Table 2
Population genetics summary statistics from three *T. dimidiata* surveys from El Carrizal, Jutiapa, Guatemala.

Fst	El Carrizal						
	Pre-spray	Early Post-spray	Final post-spray	F_{IS}	Pi	Het_{Ex}	Het_{Ob}
Pre-spray	0	0.0057	0.0053	0.290	0.065	0.065	0.034
8 months post-spray		0	0.0085	0.154	0.058	0.057	0.033
22 months post-spray			0	0.204	0.064	0.064	0.032

used in this study were successful at resolving kinship patterns at the household, local, and village scale, allowing a greater precision of source assignment than was possible previously. In the absence of insecticide treatment, individual houses displayed relatedness patterns consistent with multigenerational, extended families (Figs. 4A, 6A,B). Further supporting the conclusions of within-house breeding, ~70% of the sampled population in all surveys of both El Carrizal and El Chaperno were comprised of nymphs, which are capable of dispersal but move less than adult males (Monroy et al., 2003). Although these results may overestimate the true proportions given that nymphs are generally found in groups while adults are mostly solitary (Zeledón et al., 1970a; Bustamante et al., 2009; Monroy et al., 2009), nymphs may be indicative of successful multi-generational establishment within houses. The genetic distinctiveness of individual houses matches the results of Stevens et al. (2015) in Guatemala, which found significant genetic differentiation among houses using microsatellite markers, and suggests that previous work suggesting panmixia among houses and villages in the neighboring Department of Santa Rosa may have been hampered by low marker resolution (e.g., Dorn et al., 2003). It is in sharp contrast to the demographic composition and population dynamics of infested houses in the Yucatan, however, which match expectations of high immigration and low within-house fecundity (Gourbière et al., 2008). Differences may reflect different levels of domestic adaptation in the two regions (Waleckx et al., 2015); the Yucatan includes a cryptic species identified through gene sequencing and genome-wide SNP data (Dorn et al., 2016; Justi et al., 2018). Although most co-habiting insects were related, sibling/parental kinship levels (k = 0.5) were extremely rare (< 1%) and limited to the early post-spray recovery period, supporting the conclusion by Melgar et al. (2007) that infestations are derived from multiple unrelated founders, either during initial colonization or from subsequent immigration.

Despite low average relatedness, nearly 50% of insects collected from sprayed houses during the two post-spray surveys were found to

Table 3
Population genetics summary statistics from two *T. dimidiata* surveys from El Chaperno, Jutiapa, Guatemala.

Fst	El Chaperno					
	Pre-dispersal	Post-dispersal	F_{IS}	Pi	Het_{Ex}	Het_{Ob}
Pre-dispersal	0	0.0054	0.304	0.072	0.072	0.034
Post-dispersal		0	0.294	0.070	0.070	0.034

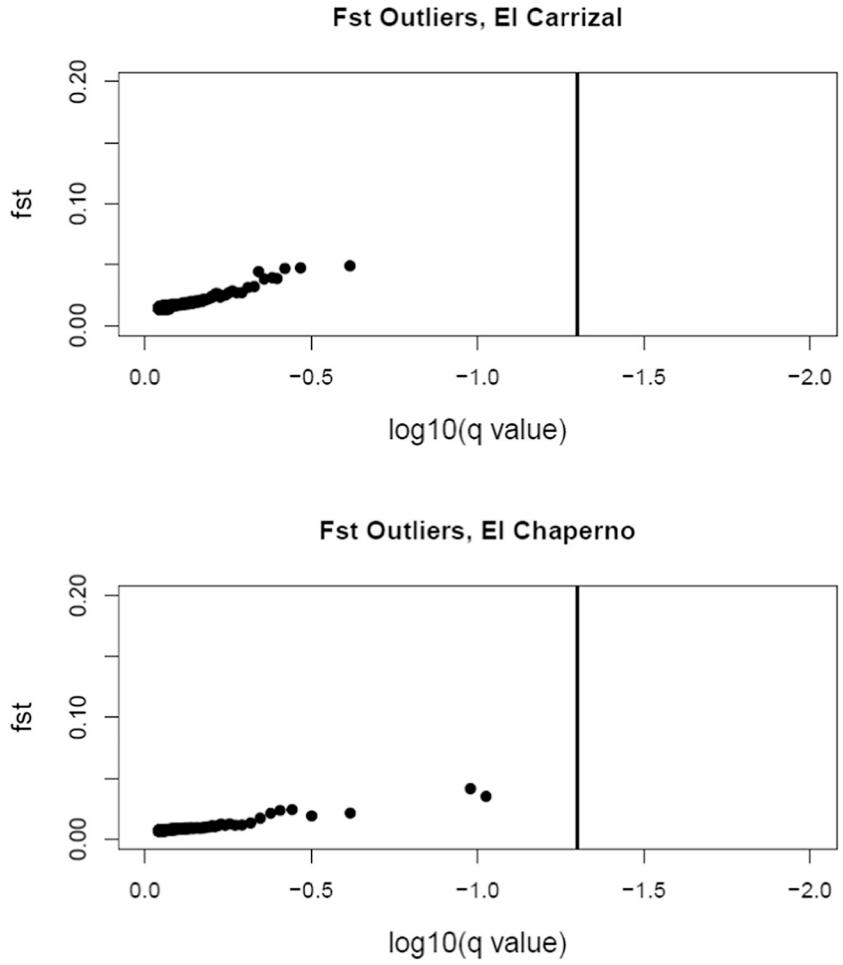

Fig. 7. Plot of results of Bayescan Fst outlier test for the villages of el Carrizal and El Chaperno. For each village, the relationship between per-locus Fst across time points and the log(10) q value is displayed; each point represents the result for a single SNP. Vertical line indicates the threshold FDR of 0.05, with significant loci falling to the right of the line.

be most closely related to pre-spray insects from the same house, more than twice the proportion expected if reinfestation had occurred at random. Two processes may explain this pattern. First, insecticide application may fail to thoroughly eliminate specimens inside houses, allowing surviving individuals to persist and reproduce. These findings are consistent with direct observation that a single insecticide treatment eliminates < 20% of domiciliated insects in a house (Monroy et al., 1998b). Houses whose construction makes them susceptible to *T. dimidiata* infestation are characterized by the presence of physical refuges where insects can hide and possibly escape insecticide contact, including absent or cracked wall plaster, unfinished floors and roofs (Bustamante et al., 2009, 2014; Sol Gaspe et al., 2015). Eggs are also known to be resistant to insecticide (Zeledón et al., 1970a), and evolved resistance to pesticides has been reported in related triatomines as a result of sustained eradication programs, although resistance has not yet been documented in *T. dimidiata* (Mougabure-Cueto and Picollo, 2015). Alternatively, insects may enter vacant niches post-spray from the peridomestic environment. Peridomestic refuges, including domestic animal enclosures, wood piles, stone walls, and other structures with abundant crevices, can serve as significant insect reservoirs, and blood feeding patterns are suggestive of high levels of movement between the house interior and peridomestic spaces in both *T. dimidiata* and *T. infestans* (Bustamante et al., 2014, De Urieste-Stone et al. 2015, Gürtler and Yadon, 2015, Provecho et al., 2017).

In addition to persistence within houses, we found evidence that dispersal activity is a significant contributor to reinfestation following insecticide application. The proportion of unrelated co-habitants within houses increased significantly in the 8 month post-spray census, becoming the most frequent relatedness class at this time point (Fig. 8B). Our results suggest that dispersal is a localized process. House infestations were spatially clustered at both post-spray time points, and post-spray dispersers were derived from houses significantly closer than that expected at random, suggesting dispersal is primarily local with less long-distance migration (Fig. 5). Population genetic structure was also consistently spatially structured, with pockets of distinct clusters occupying more geographically isolated areas of the village and significant spatial autocorrelation of genetic cluster membership both prior to insecticide application and at the end of post-spray recovery, a pattern inconsistent with global influx from a larger sylvan population (Fig. 8). Short-distance movement appears to be a general characteristic of *T. dimidiata* in this region. Although El Chaperno, which did not receive insecticide application during the study period, showed no evidence of spatial autocorrelation when sampled just prior to the dispersal season, significant local autocorrelation appeared following dispersal at an even smaller spatial scale than that observed following insecticide application (Fig. 11). This conclusion is congruent with previous findings that show *T. dimidiata* to be a relatively poor flier that depends on terrestrial movement or passive transportation, implying

Fig. 8. Temporal and spatial distribution of *T. dimidiata* genetic clusters in infested houses across the village of El Carrizal, Jutiapa, Guatemala. The spatial distribution is depicted separately for the pre-spray (A), the early post-spray (B), and the final post-spray survey (C). Circles in map are sized according to the total number of specimens sequenced from each house and colored by the genetic identity of each specimen. Insert shows the DAPC plot inferred from 2780 polymorphic SNPs, where 51.7% of the variation is explained by the first two eigenvalues. Ellipses in the DAPC correspond to the 95% confidence interval of the genetic clusters.

that dispersal is most likely at short distances (Dumonteil et al., 2004; Monroy et al., 2003). Local dispersal may be a typical feature of Chagas vectors, as proximity to nearest infested house also significantly predicted infestation by *T. infestans* in Gran Chaco, Argentina (Sol Gaspe et al., 2015).

The flow of dispersers appears to be driven by local movement from high-infestation houses. This is most clearly seen in El Chaperno, where a single house (D-54) initially contained 21% of all samples forming genetic cluster 1 and was relatively distinct from nearby houses in cluster membership, but following dispersal both overall infestation incidence and cluster 1 membership increased in the area immediately surrounding this house (Fig. 10B). Low density of potential source houses post-insecticide application may explain the relatively larger spatial scale of autocorrelation in El Carrizal, as dispersers are likely to encounter few competitors for vacant niches even at higher distances from their natal source.

Our findings address one of the most limiting characteristics of chemical control: the inability to prevent population recovery after the residual effect of the insecticide has subsided. In Jutiapa, Guatemala, we observed that *T. dimidiata* populations disperse frequently to neighboring houses through seasonal dispersal activities. In combination with resident insect survival, under the right biological conditions (e.g. frequent blood meals) *T. dimidiata* can repopulate a house in only a few generations. The relative importance of these recolonization routes

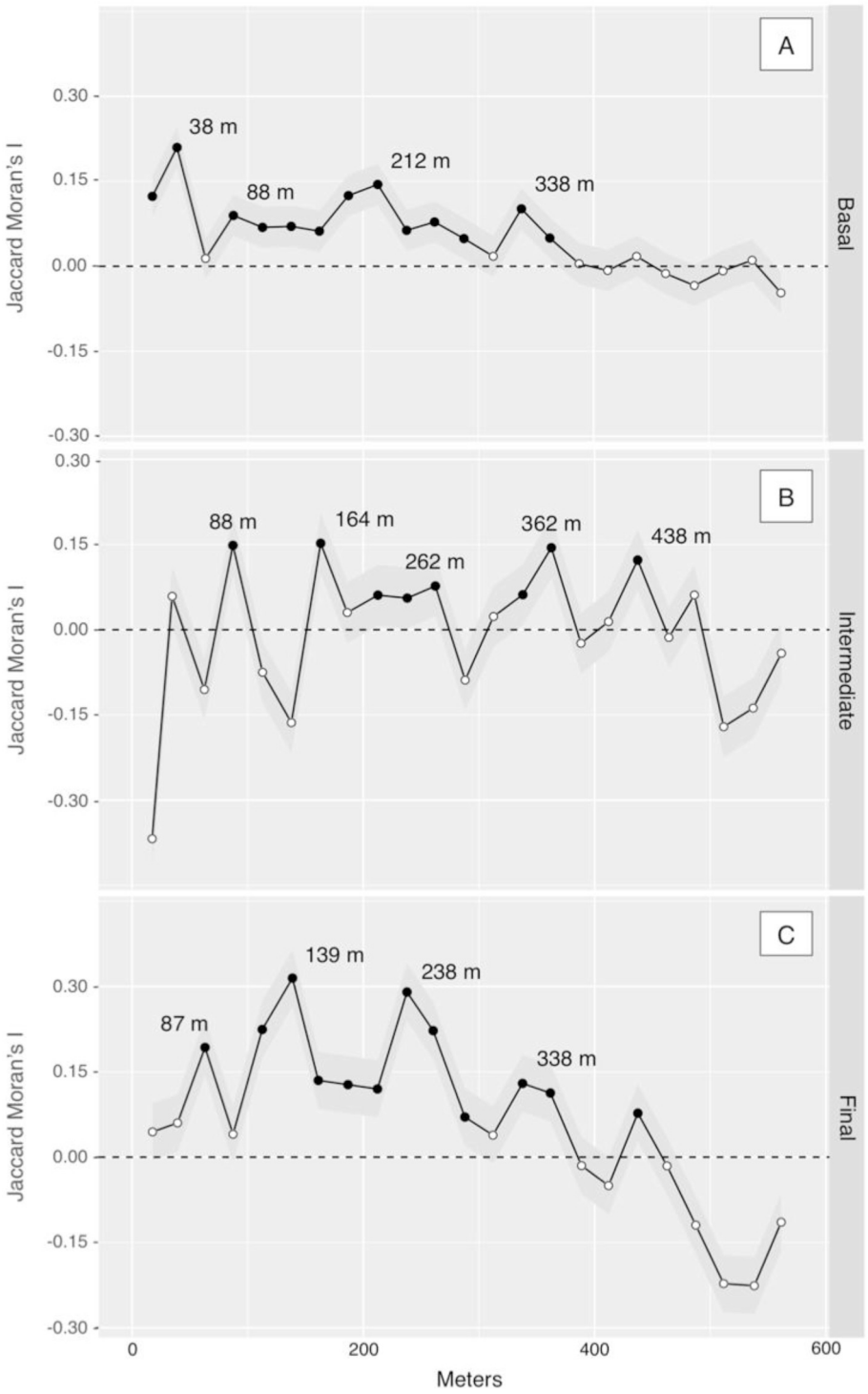

Fig. 9. Correlograms of the spatial autocorrelation inferred from the Jaccard pairwise distance among the genetic identity of individuals within each house in El Carrizal. Moran's I was calculated from (A) pre-spray, (B) early post-spray, and (C) the final post-spray populations. Spatial distance intervals were set at 25 m. The autocorrelation window was set at 560 m as established by the maximum range of spatial autocorrelation among surveyed houses. Darker grey ribbons surrounding the trend line represent the 95% BCa confidence interval calculated from 10,000 bootstraps.

may vary in other areas due to topographic, features, spatial configuration of houses and architectural and cultural practices, all of which may alter the ease and frequency of post-insecticide survival and dispersal capacity. However, both the biology of *T. dimidiata*, a native vector known for high regional mobility (Monroy et al., 2003; Stevens et al., 2015) and the frequent incidence of rapid re-infestation (Nakagawa et al., 2003; Yoshioka et al., 2015; 2018) are suggestive that these may be common impediments to vector management. Additional studies in other geographic regions should be undertaken to determine the extent to which the results found here are generalizable to other Chagas-susceptible communities.

Although an intensive, frequently scheduled insecticide program that includes all houses regardless of direct evidence of infestation, such as that used to eradicate *R. prolixus* from Central America (Hashimoto and Schofield, 2012), could potentially permanently eliminate *T. dimidiata*, the current sporadic insecticide application only for infested

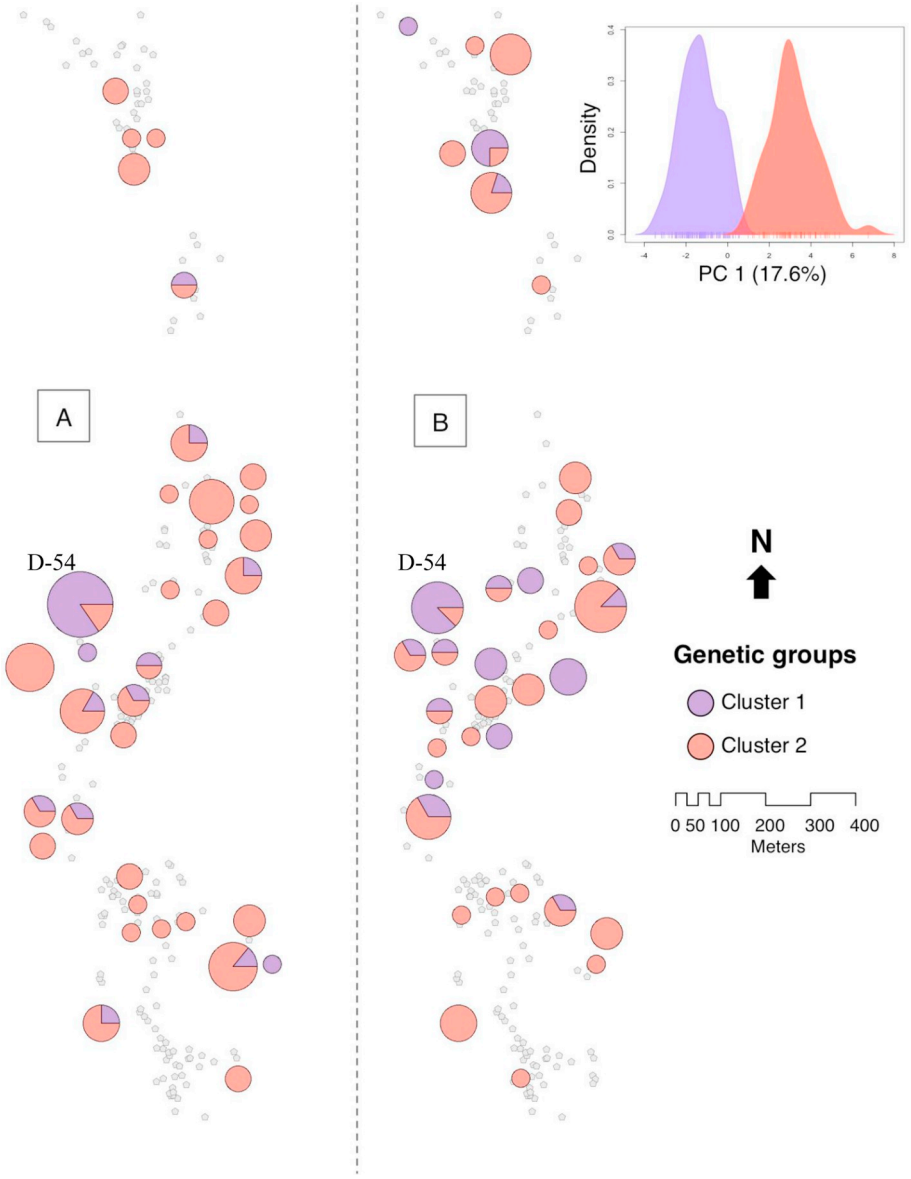

Fig. 10. Temporal and spatial distribution of *T. dimidiata* genetic clusters in infested houses across the village of El Chaperno, Jutiapa, Guatemala prior to (A) and following (B) the dispersal season. Circles in map are sized according to the total number of specimens sequenced from each house and colored by the genetic identity of each specimen. House D-54, which contained 21% of cluster 1 insects pre-dispersal, is indicated on both maps. Insert shows the DAPC plot inferred from 2260 polymorphic SNPs, where 17.6% of the variation is explained by the first eigenvalue.

houses is easily overcome by the ability of the residual population to breed and disperse as long as sufficient habitat remains available in the domestic environment. Efficacy could be improved by returning to universal spraying of houses, increasing the frequency of periodic insecticide application, as well as by adding short-term follow-up applications weeks or months following spraying to target surviving insects; however, financial pressures, limited availability of insecticide and the labor-intensive nature of insecticide campaigns may make these options difficult for ministries of health to implement. Moreover, implementing Ecohealth strategies that include both spraying and low-cost house improvements and integrated pest management can increase the long-term effectiveness of control by interrupting the process of house colonization (Cardinal et al., 2007; Gürtler and Yadon, 2015; De Urioste-Stone et al., 2015; Lucero et al., 2013; Monroy et al., 2009; Yoshioka et al., 2015; Zamora et al., 2015; Zeledón and Rojas, 2006).

5. Conclusions

Previous studies have addressed the importance of preventing dispersal from peridomestic and sylvan sources after insecticide applications; however, the improved spatial resolution made possible by high-throughput SNP genotyping technologies has significantly improved our ability to identify small-scale patterns and dispersal dynamics over time. This study is the first to show that insecticide survival and dispersal among neighboring households are crucial factors facilitating recovery of the vector in households. Explicit consideration of the role of highly-infested households and unimproved structures as reservoirs should lead to improved management and control of the vector in these highly vulnerable communities.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.104000.

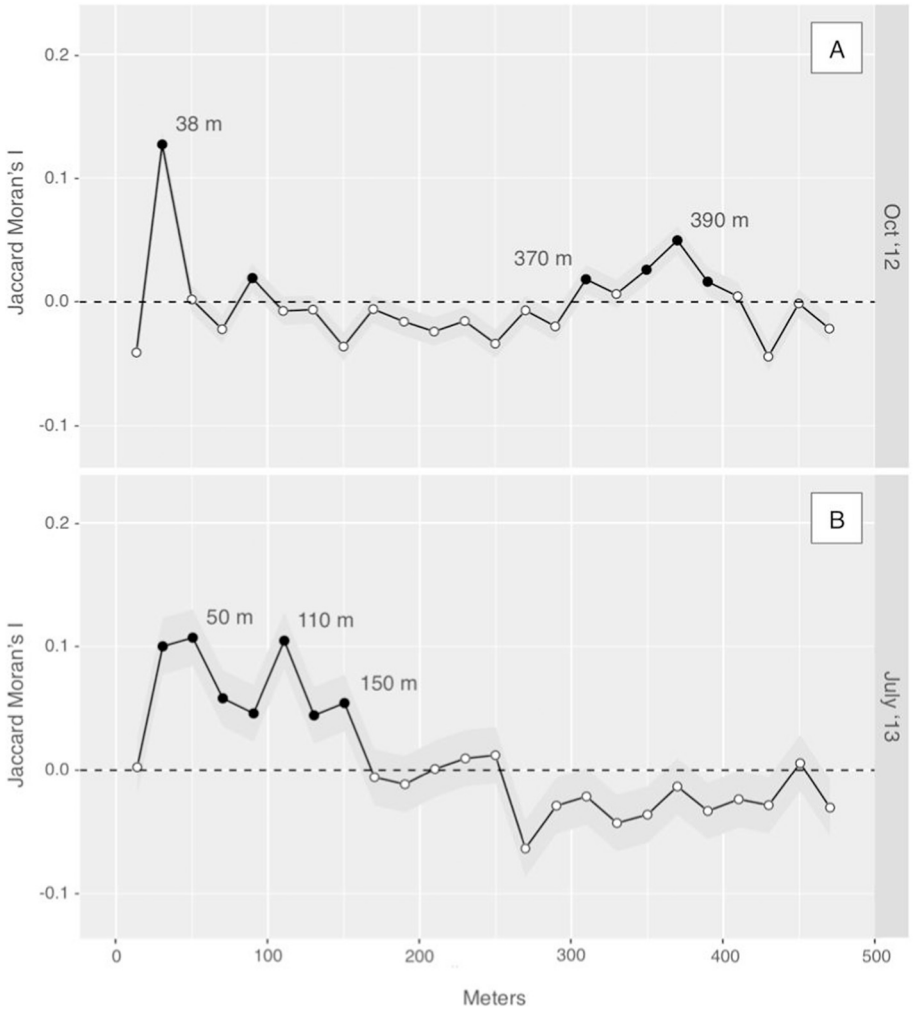

Fig. 11. Correlograms of the spatial autocorrelation inferred from the Jaccard pairwise distance among the genetic identity of individuals within each house in El Carrizal. Moran's I calculated from (A) pre-dispersal season, and (B) post-dispersal season populations. Spatial distance intervals for the pairwise comparisons were set at 20 m. The autocorrelation window was set at 460 m as established by the maximum range of spatial autocorrelation among surveyed houses. Darker grey ribbons surrounding the trend line represent the 95% BCa confidence interval calculated from 10,000 bootstraps.

Data accessibility

Sample metadata, matrices of pairwise household distances and SNP genotypes for all samples in both villages, in Variant Call Format, are available at https://github.com/shelmscahan/IGE_HelmsCahan_2019. Precise coordinates of individual houses are not provided, in order to maintain the privacy and anonymity of homeowners.

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