# INVESTIGATING MITOCHONDRIAL DNA GENES AND BIOASSAYS OF LANTANA LACE BUG TO ASSESS A POSSIBLE HOST CHANGE

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## ABSTRACT

Understanding the causes of non-target host attacks by biological control agents is essential for improving the predictive power of host-specificity testing for insects. Testing host shifts can be challenging when rare instances of non-target attacks occur. The investigation of genetic variation within different agent populations paired with feeding and reproduction bioassays can provide a better understanding of control agent host-specificity when control agents display rare behaviors of attacking a species distantly related to the original host. *Teleonemia scrupulosa* (lantana lace bug) was first introduced to the Hawaiian Islands in 1902 as a biological control agent for the invasive plant, *Lantana camara* (lantana) and helped successfully control lantana throughout the Hawaiian Islands. Years later *T. scrupulosa* was reported feeding on a distantly related endemic plant species, *Myoporum stellatum*, in the absence of lantana. To better understand this rare case of non-target attack, insect-plant host bioassays and the investigation of intraspecific genetic variation of mitochondrial DNA using three different lantana lace bug groups from two Islands were conducted. Results show slight genetic variation of 3 haplotypes within one *T. scrupulosa* group on Hawaii Island, but overall, no statistically significant variation among the three groups. Bioassay results indicate the Oahu group is more fit to utilize not only *M. stellatum* but also *M. sandwicense*. The overall bug fecundity was higher on the *Myoporum* species among the Oahu group when compared to the West Hawaii group. The results showed the West Hawaii group was more successful at utilizing lantana than the *Myoporum* species but was still able to utilize the non-hosts. The West Hawaii and the Oahu group fecundity was statistically the same when compared on *Lantana.*

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# INTRODUCTION

Due to the introduction of non-indigenous species, native ecosystems around the world are being altered. Invasive plants can displace and outcompete successional native plants, resulting in a reduction of biodiversity in native habitats due to the invasive species' efficient dispersal and rapid establishment (Day & Zaluki 2009). Chemical and biological control are the two most common management practices used against invasive weed species. Chemical control is effective, but its potential for detrimental effects on the environment has led to an increase in use of biological control methods, which can have the advantage of long-term effectiveness (Louda et al. 1997).

Insects as a taxonomic group are extremely diverse and occupy niches of specialized and generalized diets by feeding on plant, fungi and animal types. Plant characteristics (e.g., shape, surface texture, and scent) determine the probability of being colonized by herbivorous insects (Bruce et al. 2005; Le Guigo et al. 2012; Bernays & Chapman 1994). Plant characteristics such as volatile chemicals play a significant role in host selection by repelling or attracting insects and can be sex specific (Bruce et al. 2005). Females of many insects choose host plants for oviposition and feeding in response to volatile compounds or physical contact with non-volatile compounds on plant surfaces (Webster et al. 2008; Li et al. 2017). If a non-host plant is exposed to or exhibits the same chemical signals as the host plant, a female may respond to it as a favorable host, even though the non-host plant is less suitable (Li et al. 2017).

## *Biocontrol*

Biological control (biocontrol) is the use of a specialized natural enemy (predator, parasitoid or pathogen) in a new habitat for the purpose of regulating the establishment and

dispersal of an introduced pest species. Insect species used for biocontrol typically occur in their original environment as the co-adapted consumer of a plant, in the case of weed biocontrol, or insect species, in the case of insect pest biocontrol (Hoeschle-Zeledon et al. 2013). Due to the uncertainty of the introduced biocontrol agent's establishment and success at controlling a pest species in a foreign ecosystem, tests and research are necessary to choose a control agent that has the least likelihood of causing additional ecosystem changes unrelated to the control of the pest species. For example, observations of an insect biocontrol agent in the environment of origin and surveys of surrounding vegetation for alternate hosts utilization provides information regarding host-range. Surveys of potential plant hosts in the new habitat and contained host-specificity tests using plant host from the area of origin and the potential release area also provide host-range information (Marohasy 1998; Heard 2000). Overall, a general understanding of plant-insect interaction is essential to predict how an insect biocontrol agent will behave in the new environment.

In 1858, *Lantana camara* (Verbenaceae), commonly known as lantana, was introduced to the Hawaiian Islands as an ornamental plant. The woody, perennial shrub originates from the tropical and subtropical areas of Central and South America and tolerates a wide range of climatic conditions with flowering events occurring year-round (Thomas & Ellison 1999). Fifty years after the introduction to Hawaii, lantana was designated as an invasive weed occupying large areas of pastureland and dry mesic native forest. Without any natural enemies to reduce growth or reproduction, lantana posed a major threat to agricultural and native ecosystems (Davis et al. 1992).

Lantana populations are currently suppressed in most areas in Hawaii by means of a variety of biocontrol agents (Table 1), however, *T. scrupulosa* has been reported feeding on an

unrelated plant*, Myoporum stellatum* (Scrophulariaceae) (Webster) O. Degener and I. Degener since 1954 (Maehler 1954). In 1902, *Teleonemia scrupulosa* (Hemiptera: Tingidae), commonly known as lantana lace bug, was introduced to the Hawaiian Islands from Mexico for biocontrol of lantana. Additional introductions of *T. scrupulosa* from Brazil, Honduras, and Trinidad took place throughout the Hawaiian Islands in 1954 (Krauss 1961).

In 1902, host-specificity tests were not conducted, and the decision to release *T. scrupulosa* was based solely on field observations (Swezey 1924). Host-specificity tests are essential for predicting host selection behaviors of candidate biocontrol agents prior to their release. Host-specificity tests involve extensive research designed to test the possibility of non-host utilization. By using plants related to the known host, host-specificity tests create a scale of the likelihood a biocontrol would attack a species other than the host found in the new region (Marohasy 1998; Heard 2000). There are two general types of host-specificity tests: no-choice tests expose the insect to only one plant species at a time, forcing a narrow range of behaviors and outcomes; and choice tests allow the insect to select and consume from a range of plants, presumably revealing relative preferences. Within these two types of tests, there are multiple variations, including: cage, no-cage and sequential tests (Marohasy 1998).

The case of a non-target host shift from lantana to *M. stellatum* (locally known as naio) has been recorded on the island of Oahu where this particular species of *Myoporum* is endemic. There are other species of *Myoporum* found throughout the Hawaiian Islands and none have been reported to be utilized by the lantana lace bug other than *M. stellatum*. Throughout the Hawaiian Islands *Myoporum spp.* occupy elevational gradients from sea-level to 3000 m on mostly leeward slopes (Webster 1951). *Myoporum* is also extremely polymorphic, with shrub and tree growth forms, variable leaf sizes, and pubescent or glabrous surfaces (Webster 1951; Rock 1913).

In Uganda, lantana lace bug was introduced as a biocontrol for lantana in the early 1960s. When populations of the insect became high, *T. scrupulosa* infested *Sesamum indicum* (Pedaliaceae) causing some damage and reduction in yield (Davies & Greathead 1967). This event is thought to have occurred because of the increase in the *T. scrupulosa* population and decreased lantana foliage (Marohasy 1998). *Teleonemia scrupulosa* was found feeding on *S. indicum* as an alternate food source and reproducing to a very limited extent (Davies & Greathead 1967; Pemberton 2000). This event could be due a spillover effect where a predator species migrates and utilizes a different prey species (Rand et al. 2006; Chalak et al. 2010). This non-target occurrence does not confirm a host shift of the biocontrol due to the limited ability to utilize *S. indicum*. This case therefore only brings to light an expanded host range showing *T. scrupulosa* is not as specialized as originally perceived.

*Genetic Variation*

Intraspecific genetic variation within phytophagous insects may help explain observed variation in host range. Scientists have found insect populations will change reproductive and feeding behaviors when the preferred host is low in abundance (Bernays and Graham 1988). *Teleonemia scrupulosa* attacking the non-target Hawaiian native plant, *M. stellatum,* is possibly an example of genetic variation within the insect species being expressed as different populations utilize alternative hosts. This variation type can lead to shifts in host-specificity due to various levels of alternative host utilization within a population (i.e. increased adaptive potential within the biocontrol agent) (Sheppard et al. 2005; Li et al. 2017). An indicator of such variation is a shift in host-specificity in a separate population for a non-target host while the biocontrol species as a whole uses and is specific to the target host only (Sheppard et al. 2005). Genetic variation within *T. scrupulosa* populations in Hawaii may be substantial, since there were multiple

introductions from various regions, allowing for a possible genetic basis for a shift in host-specificity.

The overall purpose of this study was to examine the relationship between *T. scrupulosa* and *M. stellatum* by investigating physiological and molecular variables that may be associated with alternative host utilization. To do this, we conducted bioassays investigating aspects of physiological mechanisms of *T. scrupulosa* host utilization, by recording oviposition and development rates. We also looked for haplotype differences in the mitochondrial DNA (mtDNA) of *T. scrupulosa* from three different geographic locations and compared haplotypes of insects found on *M. stellatum* and lantana. This study provided preliminary data to be used to understand how and why the observed host shift occurred and assess whether this type of shift can be predicted in future host testing.

## MATERIALS & METHODS

*Study Species*

*Teleonemia scrupulosa* have a short life cycle of about 30 days and are native to Central and South America (Day et al. 2003). Adult females partially insert individual eggs into veins on the underside of leaves, and both adults and nymphs feed on cell contents causing necrotic lesions, deformed leaves, and defoliation (Day et al. 2003, Khan 1944). Eggs hatch within 8 days after being laid, and the nymphs matured to adulthood within 14 days when reared in a greenhouse with natural lighting and average temperature of 23.9°C. For this study, insects collected from Hawaii Island and Oahu (Table 2 and ) were reared on originating plant species to minimize behavioral variability. Thus, insects collected from lantana on Hawaii Island were reared on lantana, and insects collected from *M. stellatum* on Oahu were reared on *M. stellatum.*

*Myoporum stellatum* is endemic to Oahu lowland areas and grows as a shrub with pubescent leaves and stems. Since *M. stellatum* is endemic to the Kalaeloa region of Oahu, cuttings were collected from this area then wrapped in moist cloth and transported in resealable plastic bags. *Myoporum sandwicense* found on Hawaii Island grows in tree or shrub form. Cuttings of the low growing shrub variety of *M. sandwicense*, also known as naio papa, were used for this study. The variety of lantana used was the wild form with spiky pubescent hairs on the leaf surface and thorny stems. All cuttings were propagated using rooting powder (0.1% Indole-3-butyric acid) and kept on a mist bench until substantial roots were visible from the underside. All cuttings and seedlings collected were propagated in Hilo, Hawaii Island.

*Genetics*

Thirty-seven maternally inherited genes make up the mtDNA of insects (Clary & Wolstenholme, 1985; Crozier & Crozier 1993; Mitchell et al. 1993). Among those genes are cytochrome c oxidase subunit I and II (*COI* and *COII*), which are widely used for insect population genetics (Simon et al. 1994). Cytochrome c oxidase subunit I and II were selected for this study because previous research using these genes resulted in the ability to identify variation within insect populations (Muir and Price 2008; Guilbert et al. 2014; Yang et al. 2017). DNA extractions from freezer stored *T. scrupulosa* were performed using the QAIGEN DNeasy blood and tissue kit protocol (QIAGEN Inc., Valencia, CA) which included 24 hours digestion of tissue using Proteinase K with resulting volume of >150 µl DNA solution (Qiagen Inc., Hilden Germany). Two locus-specific primer pairs were used for amplification: C1-J-2183 CAA CAT TTA TTT TGA TTT TTT GG, TL2-N-3014 TCC AAT GCA CTA ATC TGA CAT ATT A (Simon et al. 1994) and C1-J-2798 CCW CGW CGW TAY TCW GAY TAT CC, C2-N-3554 GTT CAT GAR TGW ARD ACA TC (Damgaard et al. 2000). Polymerase chain reaction (PCR) amplifications were conducted in a 25µl reaction volume containing 1µl template; 1µl of 0.4 µM for each primer; 9.5µl ddH2O; and 12.5µl Q5 Hot Start High-Fidelity 1X Master Mix (New England BioLabs). PCR cycling conditions using Mastercycler Pro thermal cycler (Eppendorff) included 98°C preheat, 98°C for 30 s initial denaturation, 30-40 cycles of 98°C for 10 s denaturation, 48°C for 1 min *COI* annealing, 50.1°C for 1 min, *COII* annealing, and 72° C for 23 s extension. Final extension at the end of amplification was set at 72°C for 2 min.

PCR-product was tested using GelRed$^{TM}$ nucleic acid dye, 1.5% agarose gel, and sized against 1kb DNA ladder (Promega) under UV-light. PCRs were then cleared of primers and unused nucleotides with GeneJET Gel Extraction kit (Thermo Scientific). PCR reactions were

sequenced by the University of Hawaii Evolutionary Genomic Core (https://hilo.hawaii.edu/depts/epscor/) and Advanced Studies in Genomics, Proteomics and Bioinformatics (https://www.hawaii.edu/microbiology/asgpb/) sequencing facilities. The forward and reverse (5` and 3` ends) sequences for each sample were aligned manually and trimmed to equal lengths using BioEdit 7.0.9 (Hall 1999). Any samples with incomplete sequences, not verified by forward and reverse sequence unanimity, were excluded from analyses.

A haplotype network of the statistical analysis of *T. scrupulosa* genetic variation was created using PopART and TCS 1.2.1 (http://popart.otago.ac.nz and Clemet et al. 2002). The genetic structure of the lace bugs from two regions on Hawaii Island and one region on Oahu were examined using analysis of molecular variance (AMOVA) in Arlequin 3.5.2 (Excoffier et al. 2005) to investigate if there is variation among the three groups. Fu's F and Tajima's D neutrality test, population pairwise, nucleotide diversity were also calculated using Arlequin including ratios of nucleotide composition, number of variable sites, and haplotype diversity.

*Bioassays*

A no-choice test using lantana, *M. sandwicense,* and *M. stellatum* was conducted with *T. scrupulosa* collected January-February 2019 through a series of plant beats from wild lantana on Hawaii Island and *M. stellatum* on Oahu Island. Oviposition was recorded by counting the number of nymphs and unhatched eggs two weeks after mated pairs were caged on a growing plant shoot. A two-week timeframe ensured females were mature and capable of laying viable eggs, and 1$^{st}$ instar would hatch after the female insect deposited eggs into the leaf. The oviposition experiment was replicated five times with two pairs of males and females caged per replicate.

Suitability of *M. stellatum*, *M. sandwicense,* and lantana for development of *T. scrupulosa* from different source populations was evaluated in another no-choice experiment. The development data looking at survival were recorded 21 days after ten 1st – 2nd instar nymphs were caged on a growing plant shoot. This experiment was replicated across five caged shoots per plant species, with a set of ten nymphs per cage. Experiments were conducted at University of Hawaii at Hilo, Hilo, Hawaii Island in a controlled room with a photoperiod of 16:8 (L:D) using 24-Watt Fluorescent Grow Light Fixture bulbs with average of 23.5°C and 51.4% RH.

Nymph development into adults, number of eggs laid, number of nymphs hatched, and overall fecundity on *M. stellatum*, *M. sandwicense*, and lantana were analyzed using a two-way analysis of variance (Two-Way ANOVA) to determine variance among and between the two groups of lantana lace bug. Two-Way ANOVA was subjected to a post-hoc Tukey analysis to determine within group differences if the results were significantly different ($p < 0.05$). All analyses were performed using R version 3.4.4 statistical software (R Core Team 2017)

## RESULTS

*Genetics*

*COI* sequence data from 52 individuals and *COII* sequence data from 51 individuals were included in the analysis. Sample sizes were the results of variable sequence success rates. Eleven polymorphic sites were observed within the 716 bp of *COI* and 13 polymorphic sites within 540 bp of *COII*. Three haplotypes were defined in the partial sequences analyzed of *COI* and 2 haplotypes in *COII*. Analysis of molecular variance (AMOVA), haplotype diversity (*h*), nucleotide diversity ($\pi$), test of selected neutrality (Fu's F and Tajima's D) sequences, haplotype networks, and $\Phi_{ST}$ matrices statistics were generated for *COI* and *COII* (Tables 3-4, 7-10). Collection sites (W. Hawaii, E. Hawaii, and Oahu) were defined units in the case of selective neutrality test and diversity.

Low levels of haplotype diversity were observed in both *COI* and *COII* genes. A total of 3 haplotypes were observed among 2 individuals from the collection site groups. Haplotype diversity was skewed, with two individuals from W. Hawaii possessing different haplotypes from the primary (*COI h* = 0.2157 +/- 0.1241, *COII h* = 0.1176 +/- 0.1012) (Table 4 & 5). One *T. scrupulosa* expressed polymorphism at 10 base pairs in *COI* and 13 base pairs in *COII* (Figure 1 & 2). The second individual diverged from the primary haplotype at a single base pair within *COI* (Figure 1). E. Hawaii and Oahu were identical and no variation in *COI* or *COII* was found. F-statistics for *COI* ranged from 0 to1.59715 (Table 4). All *COI* $\Phi_{ST}$ values were 0 or -0.003 with more diversity existing within groups (100%) than among groups (0%) (Table 9). Overall *COI* $\Phi_{ST}$ is -0.00334 (Table 9). The results for *COII* show $\Phi_{ST}$ values were 0, -0.00369, or 0.00346 with diversity existing only within groups (100.00%) (Table 10). Overall *COII* $\Phi_{ST}$ was 0.00000 (Table 10).

Haplotype networks generated for the partial sequence within *COI* suggests that *T. scrupulosa* from Oahu, E. Hawaii, and W. Hawaii were almost identical except for 2 individuals, one differs by a single base pair and another showing 10 base pair polymorphisms (Figure 1). West Hawaii was the only group containing polymorphisms with two different haplotypes that differed from the primary in *COI*. The haplotype network for *COII* suggested similar results with even lower diversity. *Teleonemia scrupulosa* from Oahu, E. Hawaii, and W. Hawaii were almost identical except for one individual that differs by 13 base pair polymorphisms (Figure 2). This individual is the same lace bug that showed 10 base pair polymorphisms in *COI*.

*Bioassays: Development*

The development bioassay measured the differences between West Hawaii and Oahu lace bug populations survival from small nymph to adult on different plant species. Descriptive statistics show differences in means within the Oahu and West Hawaii groups (Table 12 and 14). Two-Way ANOVA results for the development bioassay showed no significant effect of plant species ($df= 2$, $F= 2.648$, $p >0.05$) or insect populations ($df= 1$, $F= 2.703$, $p >0.05$ (Table 15). However, the interaction between plants and populations was significant ($df= 2$, $F= 10.648$, $p <0.05$). Tukey's HSD (honestly significant difference) multiple comparison of means revealed which interactions between plants and populations were significantly different (Table 16). More nymphs developed into adults in West Hawaii population on lantana when compared to *M. sandwicense* ($p<0.05$) and *M. stellatum* ($p<0.05$). There were also more nymphs that matured to adults in the Oahu population on *M. stellatum* when compared to the West Hawaii population on *M. stellatum* and *M. sandwicense*.

*Bioassays: Oviposition*

The oviposition bioassay measured the differences in plant preference for oviposition between the West Hawaii and Oahu lace bug populations. Descriptive statistics show differences in means within the Oahu and West Hawaii population (Table 11 and 13). Two-Way ANOVA results for the oviposition bioassay counting successfully hatched nymphs showed there were significant differences in the number of nymphs hatched when the two lace bug populations were exposed to different plant species (df= 2, F= 7.444, $p$ <0.05), there was a difference between the two populations (df= 1, F= 39.938, $p$ <0.05), and the interaction between plant species and insect population was significant (df= 2, F= 9.661, $p$ <0.05). The total number of eggs laid was significantly different between the two populations (df=1, F= 8.026, $p$<0.05), and there was a significant interaction between plant species and insect population (df= 2, F= 11.153, $p$<0.05). However, the total number of eggs was not significantly different between the different plant species (df= 2, F= 2.372, $p$ >0.05) (Table 18) Overall fecundity was significantly different for populations (df= 1, F= 29.646, $p$ <0.05) and the interaction between plant species and insect population (df= 2, F= 13.897, $p$ <0.05) (Table 19). However, there was no significant difference in overall fecundity when lace bugs were exposed to different plant species (df= 2, F= 0.978, $p$ >0.05). Tukey's multiple comparison of means revealed which interactions between plants and populations were significantly different in regards to the number of eggs laid, nymphs, and overall fecundity (Table 20).

## DISCUSSION

Genetic diversity among lace bug populations in Hawaii might be expected to be high, given that there were multiple introductions from multiple regions. Surprisingly, genetic variability was very low, with limited haplotypic diversity. A total of three haplotypes occurred in *COI* and two haplotypes in *COII* were found among the three groups surveyed. One haplotype in both *COI* and *COII* was shared among nearly all individuals. The two different haplotypes in *COI* and were only seen in two individuals within the W. Hawaii group (one occurrence per haplotype). The haplotype with multiple polymorphisms in *COII* occurred only once and was expressed by the same individual in one of the *COI* diverse haplotypes indicating a single haplotype represented by that individual as quite genetically distinct from the majority of the population surveyed and analyzed. This leads to the conclusion that even though lantana lace bugs were introduced from multiple regions there is evidence for at least two distinct extant lineages, one of which seems to exist at a much lower density. Selective pressures may have reduced the genetic variability of the population as a whole but there is still evidence of at least two distinct genetic lineages that have many base pair differences in the genes studied. It is unclear if these differences ensured the successful establishment of the introduced species from 1902 through the 1950s. The rare haplotypes may be evidence of residual diversity from the original source populations, remaining in the maternal line at low density.

The caged no-choice bioassays proved there were significant differences within the Oahu group, West Hawaii group, and when the two groups' host utilization were compared. It appears the lace bugs from Oahu collected from *M. stellatum* oviposit on *M. stellatum* significantly more than lantana, and this population was willing to attempt to use *M. sandwicense* within the first generation of exposure. The number of eggs laid, successfully hatched nymphs, and overall

fecundity were all similar when the two *Myoporum* species were compared within the Oahu group. There was no significant difference in nymphal development when comparing the Oahu population on different plant species showing the range of host utilization of the two *Myoporum* species and lantana. The number of eggs, nymphs, and overall fecundity did not significantly differ for the W. Hawaii group during the oviposition bioassay on lantana and the two *Myoporum* species. However, the W. Hawaii group was far more successful at surviving on lantana compared to the *Myoporum* species during the development bioassay.

The comparisons of the two groups show the Oahu group was overall more successful than the W. Hawaii group utilizing the non-host *Myoporum* species. Interestingly, the Oahu source population apparently has become well adapted to this novel *Myoporum* host as overall fecundity was significantly higher on both *Myoporum* species when compared to lantana. Also, there was no significant difference in the number of nymphs maturing to adults when the two *Myoporum* species and lantana were compared. These results are consistent with the observed levels of utilization of the *Myoporum* species by Oahu and W. Hawaii populations.

## CONCLUSION

The release of insect biological control agents after host-specificity tests have been conducted has proven to be successful at controlling invasive species. An example of such a case in Hawaii is *Eurytoma erythrinae* , which provided a high level of control of *Erythrina* gall wasp within a short time after being released (Bell et al. 2013). Furthermore, non-target attacks by biological control agents are extremely rare and generally predictable due to the use of host-specificity testing (Pemberton 2000). As previously mentioned, the lantana lace bug was released without host-specificity tests, which would have incorporated tests on plants found in the region of origin and in the region of proposed introduction if modern biological control testing had been applied in 1902 or in the 1950s. The original collection sites in the South and Central America regions are unknow, and therefore we cannot know the plant community composition. But at a regional scale we can make reasonable conclusions. The lace bugs were thought to be lantana specialists, but due to the host-shift in the absence of lantana, the host range is also uncertain. What is certain is that lantana lace bugs in the Kalaeloa region on Oahu have adapted to utilize *M. stellatum* in the absence of the noxious weed, lantana, within a time frame of approximately 50-100 years (or within approximately 500-1100 generations) (Cilliers and Neser 1991). This study tested the oviposition rates and nymph development to adults of first-generation lace bugs exposed to different plants and found that there is indeed a host-shift within the Oahu population in the absence of lantana. False positive results occur in caged no-choice test when habituation occurs due to repeated contact and the insect would not normally attack the non-target in the field (Marohasy 1998; Heard 2000). Since the Oahu population have been observed and recorded attacking *M. stellatum* in the field, a false positive would only apply to the W. Hawaii population which utilized *Myoporum* species in this study to a minimal capacity.

The results of this study show that the majority of lantana lace bugs sampled are nearly identical with little intraspecific variation, with two distinct genetic types with many base pair differences but one of the haplotypes is infrequent with only one individual bug showing what is likely a remnant haplotype diversity retained from initial introduction populations either from 1902 or the 1950s. The low levels of genetic variation found in the Oahu population however were not correlated with the host-shift to a distantly related plant within a supposedly host-specific insect group since nearly every individual shared the same genetic type on both islands sampled. Further research should continue the study using later generations exposed to plants other than the field-collected host of *M. stellatum* to test if the lace bugs will become more successful on the host plant, lantana, after increased exposure of multiple generations and to test if other lace bug populations collected from lantana have higher overall fecundity on *Myoporum* species after multi-generational exposure. Since the lace bugs sampled were seemingly identical, other portions of lantana lace bug genome should be investigated and possibly mapped to identify diversity and the origin of collection. Also, functional genome analysis related to oviposition sensing and host-plant selection may be a viable method to identify the exact mechanism of this host-shift event with further study.

## TABLES

**Table 1**. Insects introduced to main Hawaiian Islands for lantana biocontrol (Davis et al.1992)

| Name | Family | Date Introduced & Origin | Est. | Site of Activity |
|---|---|---|---|---|
| *Ophiomyia lantanae* (Froggatt) | Agromyzidae | 1902 (Mexico) | yes | fruit |
| *Eutreta xanthochaeta* Aldrich | Tephritidae | 1902 (Mexico) | no | stem |
| *Epinotia lantana* (Busck) (=*Crocidosema lantana* Busck) | Tortricidae | 1902 (Mexico) | no | flower, shoot, seed |
| *Cremastobombycia lantanella* Busck | Gracillariidae | 1902 (Mexico) | no | foliar |
| *Lantanophaga* (= Platyptilia) pussillidactyla (Walker) | Pterophoridae | 1902 (Mexico) | no | flower |
| *Strymon echion* (L.) | Lycaenidae | 1902 (Mexico) | no | flower |
| *S. bazochii gundlachianus* (Bates) | Lycaenidae | 1902 (Mexico) | no | flower |
| *Teleonemia scrupulosa* (tingid) Stal | Tingidae | 1902, 1954 (Mexico, Honduras, Trinidad) | no | foliar |
| *Teleonemia vanduzii* Drake | Tingidae | 1952 (Cuba) | _ | foliar |
| *Blepharomastix acutangulalis* (Snellen) | Pyraustidae | 1953 (Mexico) | _ | foliar |
| *Octotoma gundlachi* Suffrain | Chrysomelidae | 1953 (Cuba) | _ | foliar |
| *Plagiohammus spinipennis* (Thomson) | Cerambycidae | 1902, 1954-60 (Mexico) | no | stem |
| *Octotoma plicatula* (Fabricius) | Chrysomelidae | 1954 (Honduras) | _ | foliar |
| *Octotoma scabripennis* (Guer) | Chrysomelidae | 1902, 1955-59 (Mexico) | no | foliar |
| *Aerenicopsis championi* Bates | Cerambycidae | 1902, 1955 (Mexico) | _ | stem |
| *Catabena esula* (Druce) | Noctuidae | 1955 (California) | no | foliar |
| *Langsdorfiafranddi* Hubner | Cossidae | 1955 (Mexico) | _ | roots |
| *Syngamia haemorrhoidalis* Guenee | Pyraustidae | 1956 (Cuba, Florida) | no | foliar |
| *Hypena strigata* F. | Noctuidae | 1957 (Kenya, E. Africa) | no | foliar |
| *Uroplata girardi* Pic | Chrysomelidae | 1961 (Brazil) | no | foliar |
| *Diastema tigris* Guenee | Noctuidae | 1962 (Panama Canal Zone) | _ | foliar |
| *Leptobyrsa decora* Drake | Tingidae | 1969 (Peru) | no | foliar |

**Table 2.** List of locations and plants from which *T. scrupulosa* were collected. Lace bugs at West and East Hawaii locations were found in low numbers which resulted in multiple collection points.

| Island | Collection Plant | Site | GPS | Elevation (m) | Survey Area ($m^2$) |
|---|---|---|---|---|---|
| Oahu | *Myoporum stellatum* | Pearl Harbor National Wildlife Refuge, Kalaeloa Unit | 21.299496, -158.086463 | 10 | 1,262 |
| West Hawaii | Lantana | Puu Waawaa Cinder Cone State Park | 19.800711, -155.841183 | 650 | 1,520 |
| | | | 19.801844, -155.842325 | 650 | 1,386 |
| | | | 19.801301, -155.842329 | 650 | 976 |
| East Hawaii | Lantana | University of Hawaii Agricultural Farm | 19.651048, -155.049136 | 81 | 66 |
| | | Waiakea experiment station Greenhouse | 19.646453, -155.077627 | 172 | 48 |

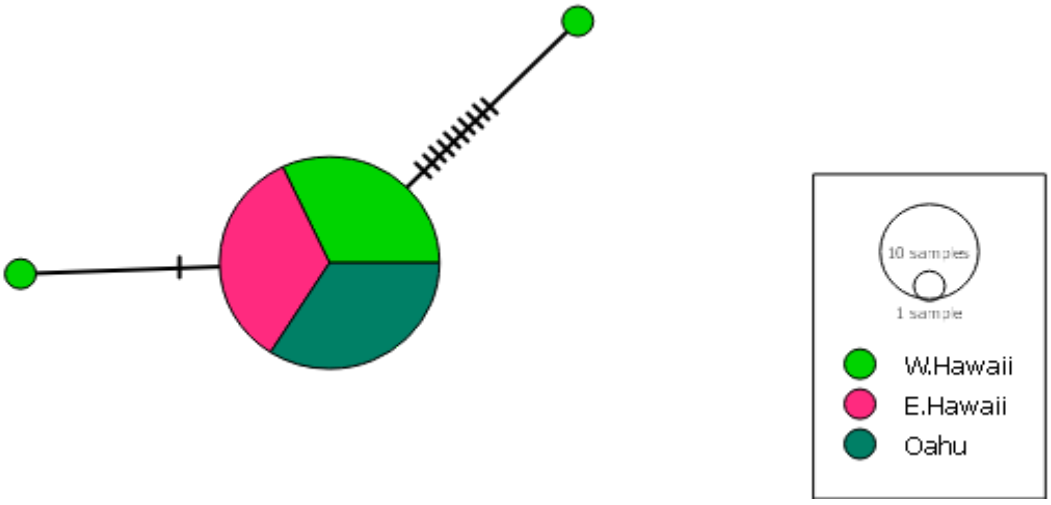


**Figure 1**. TCS haplotype network of 716 bp of the *CO1* gene region from 52 individuals collected from West Hawaii, East Hawaii, and Oahu. Three haplotypes are represented with hash marks visualizing single base pair changes. Groups are mostly monotypic apart from 2 individuals from West Hawaii.

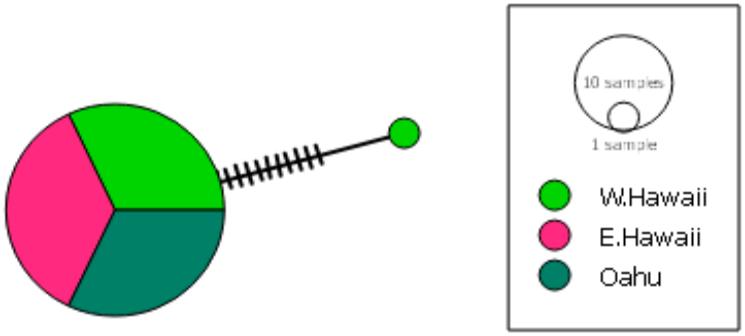


**Figure 2**. TCS haplotype network of 540 bp of the *COI1* gene region from 51 individuals collected from West Hawaii, East Hawaii, and Oahu. Two haplotypes are represented with hash marks visualizing single base pair changes. Groups are monotypic apart from 1 individual from West Hawaii with 13 bp change

**Table 3**. Genetic variability of *COI* sequences by collection sites.

| | **E. Hawaii** | **W. Hawaii** | **Oahu** |
|---|---|---|---|
| Sample size | 17 | 18 | 17 |
| Haplotype number ($N_h$) | 1 | 3 | 1 |
| Haplotype diversity (*h*) | 0 | 0.2157 +/- 0.1241 | 0 |
| Nucleotide diversity ($\pi$) | 0 | 0.001707 +/- 0.001272 | 0 |
| Tajima's D | 0 | -2.25524 | 0 |
| p value | 1 | 0.002 | 1 |
| Fu's F | 0 | 1.59715 | 0 |
| p value | N.A. | 0.84 | N.A. |

Variability of *COI* (n = 52 individuals, 3 haplotypes; 716 bp) in *Teleonemia scrupulosa* assessed in a population grouping framework. Groupings determined from collection sites. For values significance $p<0.05$

**Table 4**. Genetic variability of *COII* sequences by collection sites.

| | **E. Hawaii** | **W. Hawaii** | **Oahu** |
|---|---|---|---|
| Sample size | 16 | 17 | 18 |
| Haplotype number ($N_h$) | 1 | 2 | 1 |
| Haplotype diversity (*h*) | 0 | 0.1176 +/- 0.1012 | 0 |
| Nucleotide diversity ($\pi$) | 0 | 0.002832 +/- 0.001998 | 0 |
| Tajima's D | 0 | -2.26021 | 0 |
| p value | 1 | 0.002 | 1 |
| Fu's F | 0 | 4.03336 | 0 |
| p value | N.A. | 0.95 | N.A. |

Variability of *COI* (n = 51 individuals, 2 haplotypes; 540 bp) in *Teleonemia scrupulosa* assessed in a population grouping framework. Groupings determined from collection sites. For Fu's F and Tajima's D values significance $p<0.05$

**Table 5.** *COI* haplotype frequencies by collection site.

| | E. Hawaii | W. Hawaii | Oahu |
|---|---|---|---|
| Haplotype 1 | 17 | 16 | 17 |
| Haplotype 2 | 0 | 1 | 0 |
| Haplotype 3 | 0 | 1 | 0 |

Haplotypes of *COI* (n = 52 individuals, 3 haplotypes; 716 bp) in *Teleonemia scrupulosa*

**Table 6.** *COII* haplotype frequencies by collection site.

| | E. Hawaii | W. Hawaii | Oahu |
|---|---|---|---|
| Haplotype 1 | 16 | 16 | 18 |
| Haplotype 2 | 0 | 1 | 0 |

Haplotypes of *COII* (n = 51 individuals, 2 haplotypes; 540 bp) in *Teleonemia scrupulosa*

**Table 7.** *COI* AMOVA results

| Source of variation | d.f. | Sum of squares | Variance components | Percentage of variation | Fixation indices |
|---|---|---|---|---|---|
| Among Population | 2 | 0.4 | -0.00071 | -0.33 | |
| Within Population | 49 | 10.389 | 0.21202 | 100.33 | FST = 0.00334 |
| Total | 51 | 10.788 | 0.21131 | | |

Molecular variance is a negative value and greatest within population.

**Table 8.** *COII* AMOVA results

| Source of variation | d.f. | Sum of squares | Variance components | Percentage of variation | Fixation Index |
|---|---|---|---|---|---|
| Among Population | 2 | 0.51 | 0 | 0 | |
| Within Population | 48 | 12.235 | 0.2549 | 100 | FST: 0.00000 |
| Total | 50 | 12.745 | 0.2549 | | |

Molecular variation is only expressed within the population.

**Table 9**. *COI* pairwise $\Phi_{ST}$ values.

| | **E. Hawaii** | **W. Hawaii** | **Oahu** |
|---|---|---|---|
| **E. Hawaii** | 0.00000 | | |
| **W. Hawaii** | -0.00328 | 0.00000 | |
| **Oahu** | 0.00000 | -0.00328 | 0.00000 |

Values from *COI* sequence data.

**Table 10**. *COII* pairwise $\Phi_{ST}$ values

| | **W. Hawaii** | **E. Hawaii** | **Oahu** |
|---|---|---|---|
| **W. Hawaii** | 0.00000 | | |
| **E. Hawaii** | -0.00369 | 0.00000 | |
| **Oahu** | 0.00346 | 0.00000 | 0.00000 |

Values from *COII* sequence data.

**Table 11.** Descriptive statistics of Oahu *T. scrupulosa* collected from *M. stellatum* oviposition bioassays conducted using two mated pairs of *T. scrupulosa.* Living nymphs and unhatched eggs were counted at the end of the trial.

| | ***M. stellatum*** | ***M. sandwicense*** | **Lantana** |
|---|---|---|---|
| Total Nymph Hatch | 226 | 135 | 38 |
| Total Unhatched Eggs | 93 | 240 | 62 |
| Total Fecundity | 319 | 375 | 100 |
| Nymphs Mean | 45.2 | 27 | 7.6 |
| Eggs Mean | 18.6 | 48 | 12.4 |
| Fecundity Mean | 63.8 | 75 | 20 |
| Nymph SD | 13.6 | 19.2 | 7.5 |
| Eggs SD | 9.76 | 24.9 | 8.53 |
| Fecundity SD | 14.2 | 34.2 | 15.7 |

**Table 12.** Descriptive statistics of Oahu *T. scrupulosa* collected from *M. stellatum* development bioassay beginning with 50 nymphs (10 per cage). Nymphs that developed to adults were counted at the end of the trial .

| | ***M. stellatum*** | ***M. sandwicense*** | **Lantana** |
|---|---|---|---|
| Nymphs | 50 | 50 | 50 |
| Adults | 21 | 10 | 9 |
| Mean (SEM) | 4.2 (0.735) | 2 (0.632) | 1.8 (0.735) |
| Survival | 42% | 20% | 18% |
| Mortality | 58% | 80% | 82% |

**Table 13**. Descriptive statistics of West Hawaii *T. scrupulosa* collected from lantana oviposition bioassays conducted using two mated pairs of *T. scrupulosa.* Living nymphs and unhatched eggs were counted at the end of the trial.

| | ***M. stellatum*** | ***M. sandwicense*** | **Lantana** |
|---|---|---|---|
| Total Nymph Hatched | 15 | 2 | 27 |
| Total Eggs Unhatched | 17 | 10 | 141 |
| Total Fecundity | 32 | 12 | 168 |
| Nymphs Mean (SEM) | 3 (6.086) | 0.4 (8.579) | 5.4 (3.356) |
| Eggs Mean (SEM) | 3.4 (4.366) | 2 (11.122) | 28.2 (3.816) |
| Fecundity Mean (SEM) | 6.4 (6.336) | 2.4 (15.280) | 33.6 (7.014) |
| Nymph SD | 2.8284 | 0.5477 | 3.6469 |
| Eggs SD | 7.0569 | 3.4641 | 20.8734 |
| Fecundity SD | 9.8387 | 3.7815 | 23.6601 |

**Table 14.** Descriptive statistics of West Hawaii *T. scrupulosa* collected from lantana development bioassay beginning with 50 nymphs (10 per cage). Nymphs that developed to adults were counted at the end of the trial.

| | ***M. stellatum*** | ***M. sandwicense*** | **Lantana** |
|---|---|---|---|
| Nymphs | 50 | 50 | 50 |
| Adults | 1 | 4 | 21 |
| Mean (SEM) | 0.2 (0.2) | 0.8 (0.583) | 4.2 (1.019) |
| Survival | 2% | 8% | 42% |
| Mortality | 98% | 92% | 58% |

**Table 15**. Development bioassay Two-Way ANOVA summary.

| | df | Sum sq. | Mean sq. | F value | *p* value |
|---|---|---|---|---|---|
| Plant species | 2 | 12.8 | 6.4 | 2.648 | 0.09136 |
| Population | 1 | 6.53 | 6.533 | 2.703 | 0.11317 |
| Plant/Population Interaction | 2 | 51.47 | 25.733 | 10.648 | 0.00049* |

**p* value shows significant difference

**Table 16**. Development bioassay *Tukey's HSD* summary nymphs from Oahu and W. Hawaii lace bug populations developing into adults on *M. sandwicense*, *M. stellatum*, and lantana

| | *p* value |
|---|---|
| **Population** | |
| W. Hawaii/ Oahu | 0.1132 |
| **Plant** | |
| *M. sandwicense*/lantana | 0.0749 |
| *M. stellatum*/lantana | 0.4932 |
| *M. stellatum*/*M. sandwicense* | 0.4932 |
| **Plant:Population** | |
| *M. sandwicense*:Oahu/ lantana:Oahu | 0.9999 |
| *M. stellatum*:Oahu/ lantana:Oahu | 0.1821 |
| lantana:W. Hawaii/ lantana:Oahu | 0.1821 |
| *M. sandwicense*:W. Hawaii/ lantana:Oahu | 0.9076 |
| *M. stellatum*:W. Hawaii/ lantana:Oahu | 0.5895 |
| *M. stellatum*: Oahu/ *M. sandwicense*:Oahu | 0.2580 |
| lantana:W. Hawaii/ *M. sandwicense*:Oahu | 0.2580 |
| *M. sandwicense*:W. Hawaii/ *M. sandwicense*:Oahu | 0.8227 |
| *M. stellatumn*:W. Hawaii/ *M. sandwicense*:Oahu | 0.4661 |
| lantana:W. Hawaii/ *M. stellatum*:Oahu | 1.0000 |
| *M. sandwicense*:W. Hawaii/ *M. stellatum*:Oahu | 0.0222* |
| *M. stellatum*:W. Hawaii/ *M. stellatum*:Oahu | 0.0053* |
| *M. sandwicense*:W. Hawaii/ lantana:W. Hawaii | 0.0222* |
| *M. stellatum*:W. Hawaii/ lantana:W. Hawaii | 0.0053* |
| *M. stellatumn*:W. Hawaii/ *M. sandwicense*:W. Hawaii | 0.9892 |

**p* value shows significant difference

**Table 17**. Oviposition bioassay *Two-Way* ANOVA summary for successfully hatches nymph.

| | **df** | **Sum sq.** | **Mean sq.** | **F value** | ***p* value** |
|---|---|---|---|---|---|
| Plant species | 2 | 1566 | 783 | 7.444 | 0.003054* |
| Population | 1 | 4201 | 420 | 39.938 | 1.56e-06* |
| Plant/Population Interaction | 2 | 2032 | 1016 | 9.661 | 0.00036* |

**p* value shows significant difference

**Table 18**. Oviposition bioassay *Two-Way* ANOVA summary for number of eggs laid.

| | **df** | **Sum sq.** | **Mean sq.** | **F value** | ***p* value** |
|---|---|---|---|---|---|
| Plant species | 2 | 1015 | 507.6 | 2.372 | 0.114813 |
| Population | 1 | 1718 | 1717.6 | 8.026 | 0.009196* |
| Plant/Population Interaction | 2 | 4774 | 2387 | 11.153 | 0.000376* |

**p* value shows significant difference

**Table 19**. Oviposition bioassay Two-Way ANOVA summary for overall fecundity.

| | **df** | **Sum sq.** | **Mean sq.** | **F value** | ***p* value** |
|---|---|---|---|---|---|
| Plant species | 2 | 745 | 372 | 0.978 | 0.391 |
| Population | 1 | 11291 | 11291 | 29.646 | 1.35e-05* |
| Plant/Population Interaction | 2 | 10585 | 5293 | 13.897 | 9.80e-05* |

**p* value shows significant difference

**Table 20**. Oviposition bioassay *Tukey's HSD p* values for successfully hatches nymph, number of eggs laid, and overall fecudity from Oahu and W. Hawaii lacebug population on *M. sandwicense*, *M. stellatum*, and lantana

| | ***p* value** | | |
|---|---|---|---|
| **Population** | **Nymphs** | **Eggs** | **Fecundity** |
| W. Hawaii/ Oahu | 1.6e-06* | 0.0091959* | 1.35e-05* |
| **Plant** | | | |
| *M. sandwicense*/lantana | 0.0031* | 0.7551 | 0.3753 |
| *M. stellatum*/lantana | 1.56e-06* | 0.3461 | 0.6141 |
| *M. stellatum*/*M. sandwicense* | 0.0004* | 0.1029 | 0.9108 |
| **Plant:Population** | | | |
| *M. sandwicense*:Oahu/ lantana:Oahu | 0.0620 | 0.0090* | 0.0020* |
| *M. stellatum*:Oahu/ lantana:Oahu | 0.0001* | 0.9836 | 0.0180* |
| lantana:W. Hawaii/ lantana:Oahu | 0.9993 | 0.5401 | 0.8758 |
| *M. sandwicense*:W. Hawaii/ lantana:Oahu | 0.8725 | 0.8666 | 0.7116 |
| *M. stellatum*:W. Hawaii/ lantana:Oahu | 0.9790 | 0.9221 | 0.8758 |
| *M. stellatum*: Oahu/ *M. sandwicense*:Oahu | 0.0908 | 0.0415* | 0.9408 |
| lantana:W. Hawaii/ *M. sandwicense*:Oahu | 0.0296* | 0.3014 | 0.0280* |
| *M. sandwicense*:W. Hawaii/ *M. sandwicense*:Oahu | 0.0049* | 0.0006* | 0.0001* |
| *M. stellatumn*:W. Hawaii/ *M. sandwicense*:Oahu | 0.0126* | 0.0008* | 0.0003* |
| lantana:W. Hawaii/ *M. stellatum*:Oahu | 3.29e-05* | 0.9004 | 0.1802 |
| *M. sandwicense*:W. Hawaii/ *M. stellatum*:Oahu | 5.3e-06* | 0.4878 | 0.0006* |
| *M. stellatum*:W. Hawaii/ *M. stellatum*:Oahu | 1.35e-05* | 0.5800 | 0.0013* |
| *M. sandwicense*:W. Hawaii/ lantana:W. Hawaii | 0.9699 | 0.0862 | 0.1555 |
| *M. stellatum*:W. Hawaii/ lantana:W. Hawaii | 0.9990 | 0.1165 | 0.2725 |
| *M. stellatumn*:W. Hawaii/ *M. sandwicense*: W.Hawaii | 0.9985 | 1.0000 | 0.9995 |

**p* value shows significant difference

## APPENDICES

**Figure 3.** *Teleonemia scrupulosa* adults. Picture by M. Sheffield.

**Figure 4.** *Teleonemia scrupulosa* egg laid partially into the mid-vein of the lantana leaf**.** Picture by M. Sheffield.

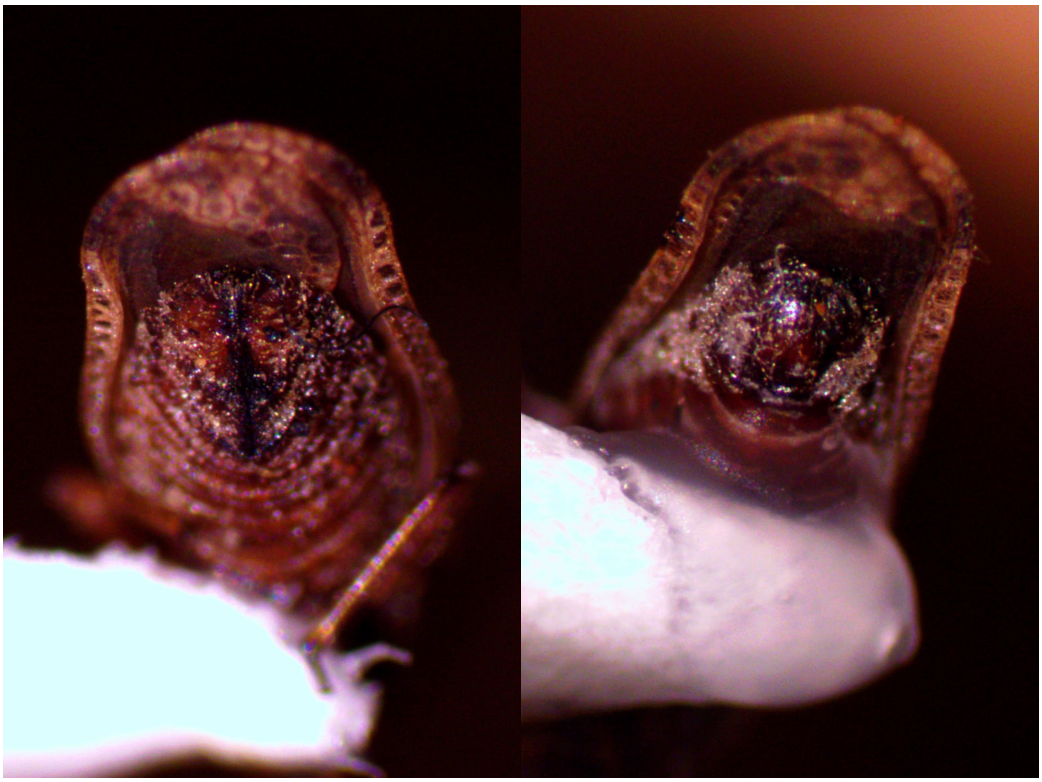

**Figure 5.** Female (left) and male (right) *T. scrupulosa*.

**Figure 6**. *Teleonemia scrupulosa* nymphs

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