**Thorne's Hairstreak (*Callophrys [Mitoura] thornei*) Monitoring**
Final report
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Matthew Forister (PI, Assistant Professor, University of Nevada, Reno; mforister@unr.edu)
Amy Lucas (Graduate student, University of Nevada, Reno)

## Abstract

This report covers the length of the project, from the summer months of 2009 to the end of summer 2012. Activities are summarized in the context of the primary goals of the project, which were completed. Our three primary objectives were (1) to document the extent of Thorne's hairstreak (TH) presence within the study area of Otay Mountain; (2) to characterize habitat association within that geographic range; and (3) to conduct larval experiments addressing the importance of tree age on the physiological performance of caterpillars. With reference to objective 1, we found the distribution of TH on Otay Mountain to be more extensive than previous reports had suggested. We also examined TH presence in the interior versus perimeter versus exterior of host plants stands. With reference to objective 2, variables characterizing vegetation and the environment were thoroughly documented but found to explain very little of the variation in TH presence/absence and abundance. Finally, larval experiments were able to definitively reject the hypothesis that older foliage might be important for larval growth. The implications of these findings for the conservation of TH are discussed. In brief, we come to the following main conclusions. (1) The widespread range of the butterfly within the study area has positive implications for persistence, though it should be remembered that the entire study area is not itself large and is prone to wildfires. (2) TH appear to associate with the host trees under a range of environmental conditions on Otay Mountain, but this should not be taken to mean that a monoculture of the trees would be sufficient for TH population persistence; to the contrary, patch edge use by TH strongly suggests that variation in patch size, area, and configuration are important and desirable targets for management and conservation. (3) Finally, we note that these findings point the way towards research that could be conducted with TH or (more likely) with closely related species to address the importance of habitat configuration and heterogeneity on population dynamics.

## Objectives

In this section, all objectives are summarized, and a brief statement of final status for each objective is given.

(1) Map Tecate cypress stands on Otay Mountain for use in designing sampling scheme, and age trees by coring.

Mapping completed in summer of 2009 (see Fig. 1). Despite two summers of collecting cores, we were never able to achieve proper preservation of the cored material that would allow us to count rings in the lab. However, given other results (such as the ubiquity of TH on the mountain, and the un-importance of tree age for larval performance), we do not believe that this outcome in any way compromised our project or the conclusions that we have reached.

2) Conduct occupancy surveys for TH adults and juveniles.

Surveys were conducted and completed in the summers of 2010 and 2011. A total of 290 individual survey points associated with 41 Tecate cypress stands were surveyed for TH adults or caterpillars.

3) Characterize habitat in terms of vegetation and environmental variables.

A total of 360 survey points were surveyed for vegetation and environmental variables.

4) Conduct larval and adult experiments to assess the importance of foliage age on adult behavior and juvenile performance.

Larval experiments were successfully conducted as planned in the summer of 2011. We were never able to conduct adult preference experiments due to limitations in the number of adults that could be caught at the same time.

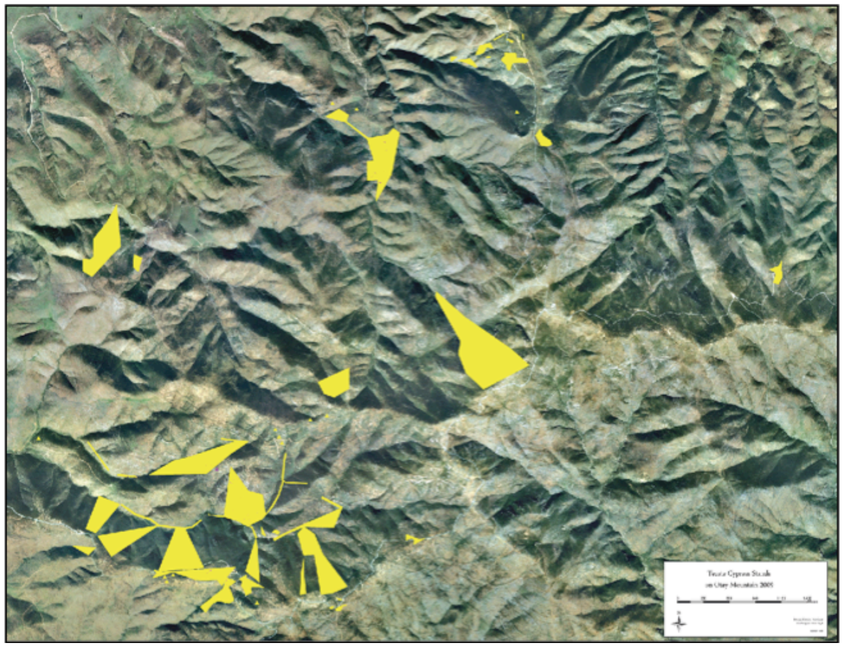


**Figure 1. Otay mountain with Tecate Cypress stands (in yellow) mapped in 2009.**

5) Analyze data and prepare final report and manuscript for publication in peer-reviewed scientific journal and as a Master's thesis.

Data analyses are complete, and are being reported here. The most important results for conservation and management are given here, while more details can be found in the Master's thesis by Amy Lucas which is on file and digitally available from the University of Nevada, Reno (the title of the thesis is "Geographic distribution, habitat association, and the importance of host quality for one of the rarest butterflies in North America: Thorne's hairstreak (Mitoura thornei)". A manuscript is currently being prepared for submission to a journal.

6) Archive all data, including Tecate cypress maps, TH survey data and vegetation survey data.

Data has been archived at the following locations: SANDAG, the USFWS office in Carlsbad, and the University of Nevada, Reno. Storage at SANDAG was facilitated by Yvonne Moore, and storage at the USFWS office by Tony McKinney. At UNR, the data is stored in the Forister lab, and queries can be directed to mforister@unr.edu.

**Introduction**

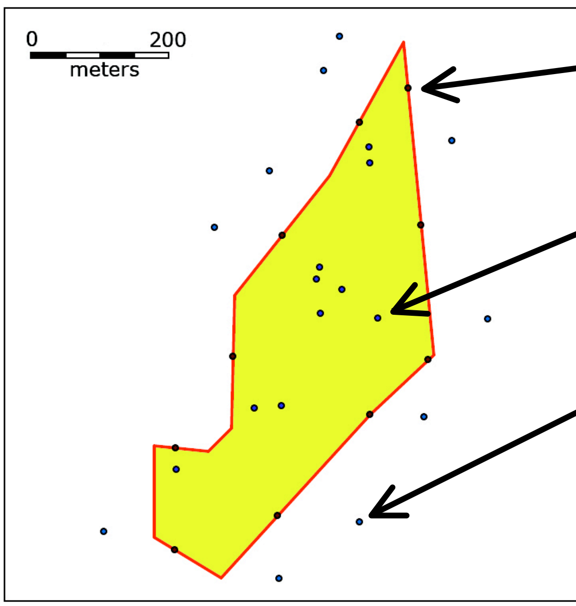
This report covers the length of the Thorne's Hairstreak Monitoring project, from the summer months of 2009 to the end of summer 2012. The project encompassed two and ½ field seasons: in 2009, we started late due to delays in the arrival of funding, and thus focused in that year only on mapping Tecate cypress stands; then in 2010 and 2011 we had full field seasons for TH and habitat surveys; then in 2012 we were not in the field but focused only on data analysis and writing. In the following sections, we provide a summary of project design, an explanation of analyses, a report on results, and finally a discussion of findings with respect to the conservation and persistence of TH.

**Project design**

The overall design has been explained in detail in previous documents submitted in association with this project. Here we offer only a brief summary for completeness in the final report. The essence of the project was semi-random sampling in space for TH and environmental variables (hereafter, "environmental" should be taken to mean variables such as slope or wind speed, as well as vegetative characteristics). Ten-meter diameter points were located randomly in Tecate stands, on the perimeter of Tecate stands and in areas immediately surrounding stands. For each stand, an equal number of points was placed in those three locations (interior, perimeter, and exterior) and the total number of points per stand was proportional to the total perimeter of the stand (Fig. 2). Note that we refer to the sampling as "semi" random only because the perimeter points were equally spaced rather than being randomly located around the perimeter. See Fig. 3 for the distribution of survey points on the mountain.

The sampling points were then visited in a random order, and were surveyed for TH adults and larvae and for environmental variables. Adults and larvae were surveyed at different times in order to maximize the amount of field time spent looking for TH (larvae can be censused when adults are not flying).

For larval experiments, eight female butterflies were taken from the field and confined in small cages with foliage. Eggs were collected from these cages, and 86 larvae were reared on foliage of three relative ages: young, medium and old. Rearing took place in the Forister lab at the University of Nevada, Reno, following protocols that the lab uses with other butterflies in the family Lycaenidae.




Perimeter points placed systematically every 200m along the perimeter from a random starting point.

Interior points placed randomly within the interior (not overlapping 5m from the edge). The number of these points is equal (when possible) to or less than the number of perimeter points.

Exterior points placed randomly within a 100m buffer around the patch (not overlapping 5m from the edge). The number of points is equal (when possible) to or less than the number of perimeter points.

**Figure 2. Exemplar patch illustrating the placement of sampling points within and around a patch of Tecate cypress (in yellow).**

**Analyses**

Our primary analyses were built almost entirely around the conceptual framework of Mantel tests, which are frequently used in ecological applications with spatial sampling. Mantel tests examine permuted correlations among distance matrices. Partial mantel tests have often been used when one is interested in the relationship between two variables (such as abundance of an organism and an environmental variable) while controlling for a third variable (such as geographic distance). Multiple Mantel tests are a fairly recently-developed extension of partial Mantel tests which allow for the examination of multiple predictor variables, and can be interpreted in a similar way to traditional multiple regression. We used both pairwise and multiple Mantel tests in a rank-based (nonparametric) framework.

Simple pairwise Mantel tests provided us with an initial screen of environmental variables predicting both presence/absence and abundance of TH. We also examined "fractional presence," which is the fraction of points at which TH were observed within a stand. Following that initial screen for significance with pairwise tests, we used multiple Mantel tests to investigate the importance of multiple variables simultaneously. Note that analyses of these different response variables were conducted at both the point and stand levels, which produced a large quantity of quantitative results. However, we found that a rather simple message emerged from the multifaceted analyses, and our results here highlight only a subset of analyses and results that are useful for understanding the core of our results.

Results from the larval rearing experiments were analyzed with simple chi-square test (for survival) and analysis of variance (ANOVA) for fresh adult weight of individuals reared on the different foliage treatments.

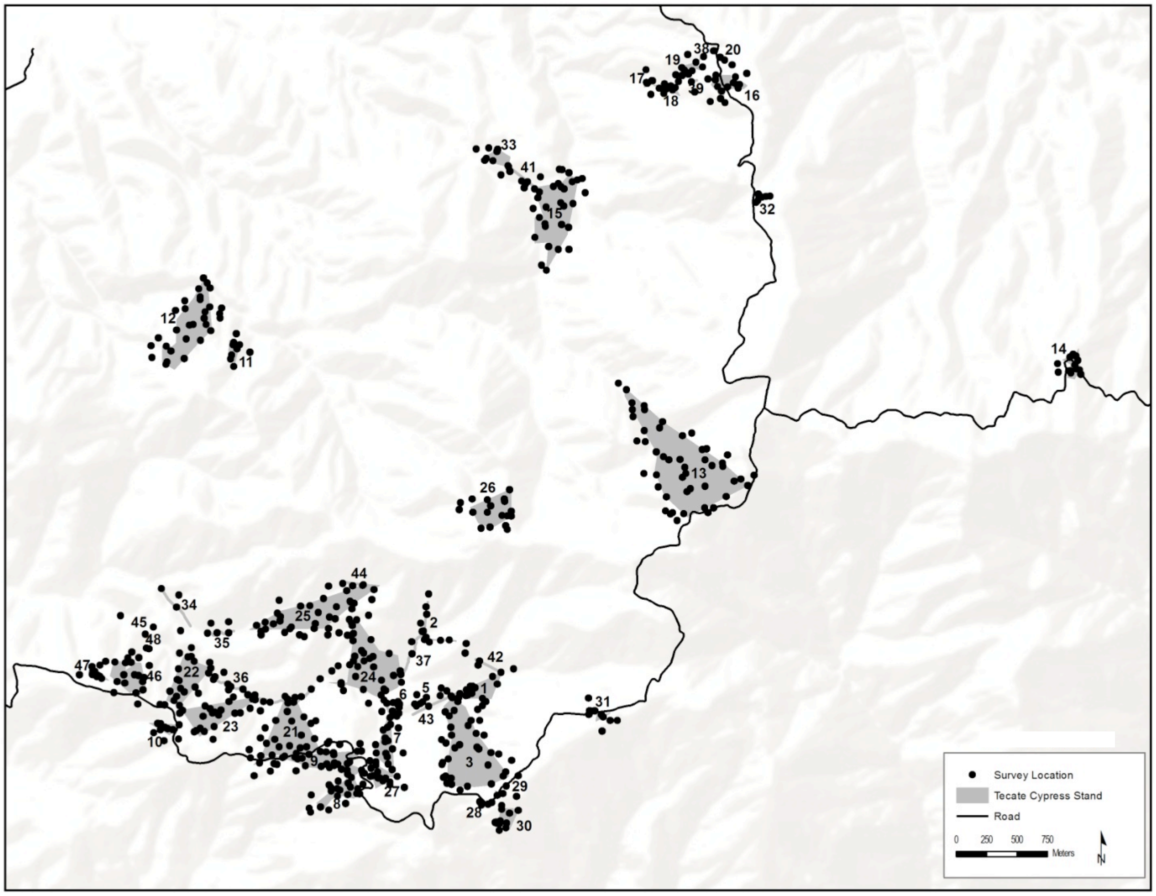



**Figure 3. Numbered stands and survey points on Otay Mountain. Each point is a survey point (624 total); stands are numbered 1-48.**

## Results

A total of 229 points were surveyed for TH adults or caterpillars, and 358 points were surveyed for environmental variables. Our total observations of TH individuals are as follows: 75 TH adults associated with 22 stands of Tecate cypress, for an average of 4.4 adults per stand (Appendix 1); 38 TH larvae were found in association with 26 points in 17 stands. Adults or larvae were found in 31 of the 41 Tecate cypress stands. Both *M. thornei* and adults were found in eight stands. See Fig. 4 for the distribution of TH sightings on the mountain, and Fig. 5 for a summary of observations with respect to the fraction of points and stands in which TH were found.

Our initial screening of environmental variables using pairwise Mantel tests revealed very few statistically significant variables. For example, at the level of individual points and presence/absence of TH, only the density of Tecate cypress and average Tecate cypress diameter at breast height (DBH) were significant at $P < 0.05$; other (non-significant) variables included: slope, herbaceous density, herbaceous richness, woody density (excluding Tecate cypress), woody richness (excluding Tecate cypress), density of grasses, size of nearest stand, and density of leaf litter, rock and bare ground. Results at the stand level (predicting the fraction of occupied points) are similar: only Tecate cypress density and Tecate cypress DBH were significant in pairwise analyses. Fig. 6 includes an illustration of bivariate relationships at the stand level for Tecate cypress density and DBH.

Table 1. Summary of results from multiple Mantel tests on presence/absence at the point level (top rows) and the fraction of points in which TH was seen at the stand level (bottom rows).

| | | F | Coefficient | P | $R^2$ |
|---|---|---|---|---|---|
| Presence/Absence | Whole Model | 282.56 | | 0.001 | 0.02 |
| | Tecate cypress density | | 0.048 | 0.008 | |
| | Tecate cypress DBH | | 0.087 | 0.001 | |
| | Distance | | -0.022 | 0.120 | |
| Fraction seen | Whole Model | 14.19 | | 0.052 | 0.009 |
| | Tecate cypress density | | 0.202 | 0.005 | |
| | Tecate cypress DBH | | 0.045 | 0.467 | |
| | Distance | | -0.037 | 0.442 | |

Subsequent to pairwise analyses, those few significant variables were combined in multiple Mantel tests for presence/absence at the point level and fractional presence at the stand level. As can be seen in Table 1, the significance of Tecate cypress density and DBH was retained in full models at the point level, while only density was retained as significant for fractional seen at the stand level. Although results are highly statistically significant, it is important to note that $R^2$ values in Table 1 are extremely low (less than 2% of variation is explained in one case, and less than 1% in the other). This result of low variation explained will be discussed further below.

We also examined the use of space by TH by comparing the density of individuals observed in the interior, perimeter and exterior of stands, and we did these comparisons separately for larvae and adults, and for larvae and adults combined. We found remarkably similar densities of individuals across perimeter and interior survey points, which can be seen in Fig. 7 (this is in contrast to the very low density of adults observed in

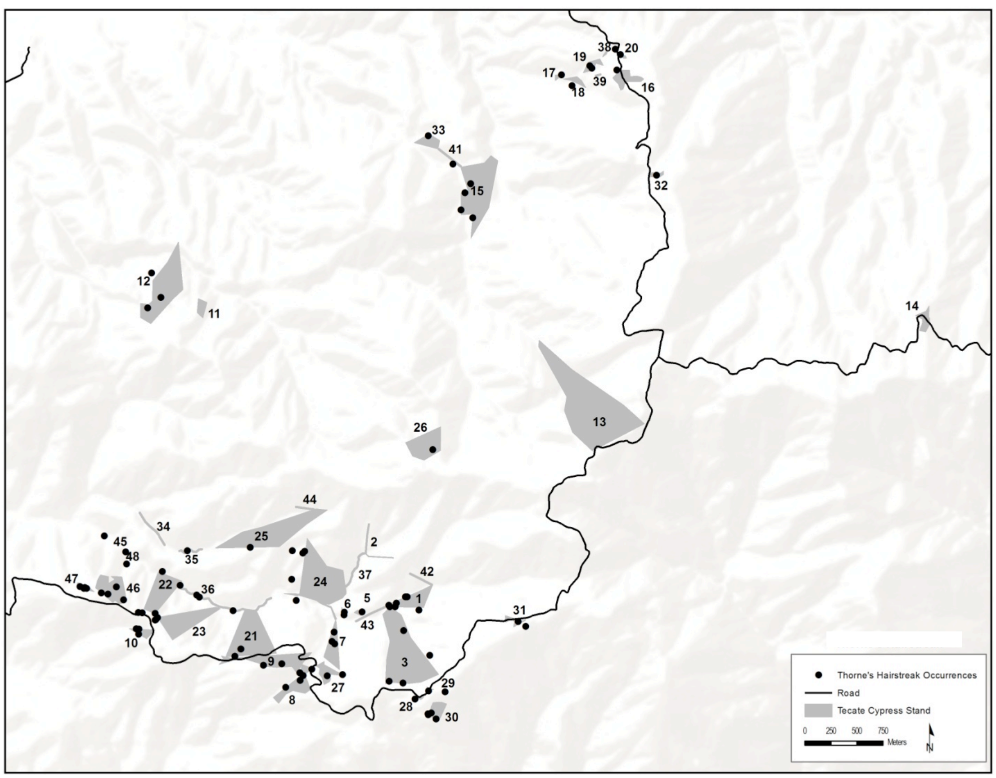



**Figure 4. Map illustrating locations where *M. thornei* was seen. Each dot represents an occurrence. Occurrence is either *M. thornei* adult or larva. Stands are numbered as in Fig. 3.**

exterior points, which is not surprising, as these points were removed from their host plants). Pairwise comparisons between density in perimeter and interior were conducted using paired Wilcoxon tests and differences were never detected (P was always > 0.05).

Finally, we addressed the physiological effects of foliage age on larval performance (Fig. 8). As we have reported in our most recent annual report, we did not find an effect of foliage age on larval survival, but we did find an effect on growth. Individuals reared on foliage from the oldest trees matured into adults that had a lower body mass than individuals reared on foliage from trees of medium and young age. The effect of foliage age is not large, but it is important to note that it is in the opposite direction of that predicted by the early suggestion in this system that older trees might be necessary for survival.

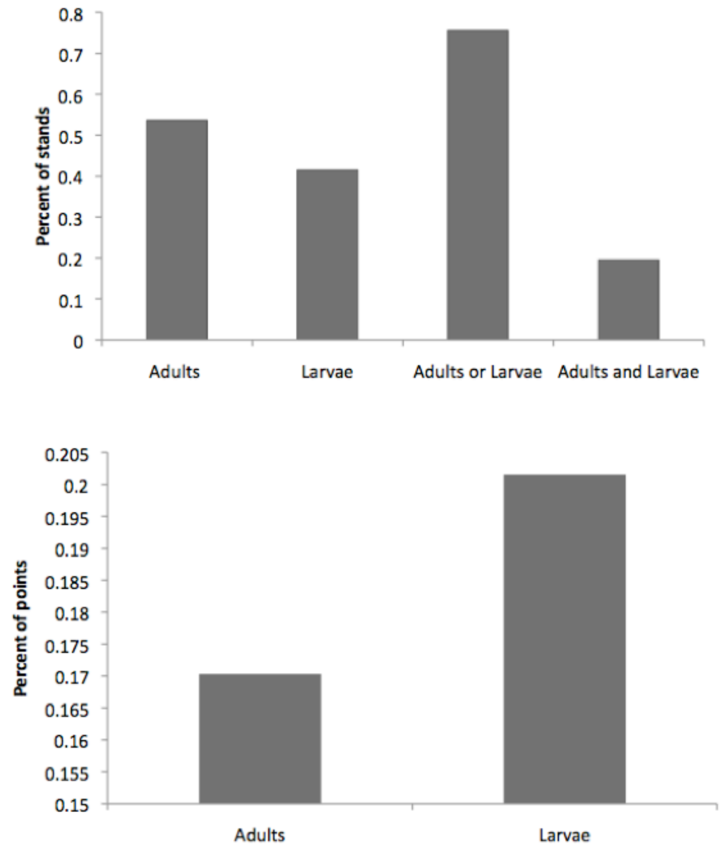



**Figure 5. Graph illustrating the percent of stands (y- axis) that *M. thornei* adults, larvae, adults and larvae, and adults or larvae were seen in (top panel) and percent of points *M. thornei* adults and larva were seen (lower panel).**

**Discussion**

It has often been the case in invertebrate conservation that surveys are confined to roadsides, which are both easy to access and easy to survey. Having a roadside-bias, however, presents serious drawbacks to our knowledge of distribution and abundance because insects (like many animals) will preferentially occupy habitat edges (which are created by roads). Our project used a randomized design that did not bias our results towards roadside observations. Consequently, our three years of extensive field work allowed us to document the widespread distribution of TH on Otay Mountain (Fig. 4). The stands in which TH was observed included both small and large stands, spread across the study area. Prior to this study, TH was known from a fraction of those stands, largely restricted to areas with immediate road access.

We measured a large number of environmental variables (biotic and abiotic) in an attempt to characterize TH habitat preferences within their range on Otay Mountain. We found that the only statistically significant predictors of TH presence were the size (DBH) and density of Tecate cypress. The positive relationship observed between TH presence in the field and DBH might be seen as contradictory to our laboratory finding that foliage from the oldest trees was the least beneficial for larval growth. However, it should be remembered that distribution in the field is very different from physiological performance. In particular, TH is a lekking species: males gather around the tops of trees and wait for passing females. This behavior could bias distribution in the field towards larger and more dense areas, which has been observed in a closely related species *Mitoura muiri* (M.L.F. pers. observation). In this light, our performance results in the laboratory should be taken as rejecting the hypothesis that older foliage might be necessary for larval growth.

Although we recovered statistically significant predictors of TH presence (Table 1), our models explained a very small proportion (2% and less) of the variance in presence/absence observed in the field. This result (low $R^2$) could suggest at least two interpretations. First, it is entirely possible that some key aspect of the biotic or abiotic environment was not measured. Second, it could be the case that variation in TH presence/absence within their range on Otay Mountain is essentially stochastic. We can not distinguish between these alternatives, which we discuss in our next section below (which also includes a discussion of perimeter/interior space use).

**Conclusions for persistence, conservation and management**

Here we provide a concise list of what we believe are our most pertinent findings with respect to the persistence, conservation and management of Thorne's hairstreak butterfly:

1) The butterfly can be considered geographically widespread *within the study area* of Otay Mountain. This is certainly good news for the persistence of the butterfly, with the following major caveats. Otay Mountain is of course not large and subject to devastating wild fires. Despite new areas of the range that we have discovered, it is easily conceivable that the entire range could be consumed by a large fire. Furthermore, within the range on Otay, the butterfly was never found to be abundant, which means that we can not hypothesize with respect to any source-sink dynamics (if they exist for this species). This lack of knowledge regarding population (or metapopulation) structure will impede any responses that might be taken in the face of a wildfire.

2) TH were found in association with Tecate cypress trees under a range of biotic and abiotic conditions. Indeed, Tecate cypress density and Tecate cypress DBH were the only significant predictors of presence. It is very important that this result not be taken to mean that a monoculture of Tecate cypress would be optimal habitat for TH. If nothing else, the butterfly needs nectar which can not be had from the host plant Tecate cypress, nor presumably can much nectar be found within dense stands of cypress. Thus the butterflies must depend on access to the matrix of non-host habitat, which raises the interesting issue of space. We found that TH individuals were equally likely to be found on the edges of patches as well as in the interior. Because our study did not explicitly address spatial issues, we can only speculate, but we believe that it is entirely possible that optimal TH habitat consists of small or medium-sized patches of Tecate hosts embedded in a matrix that

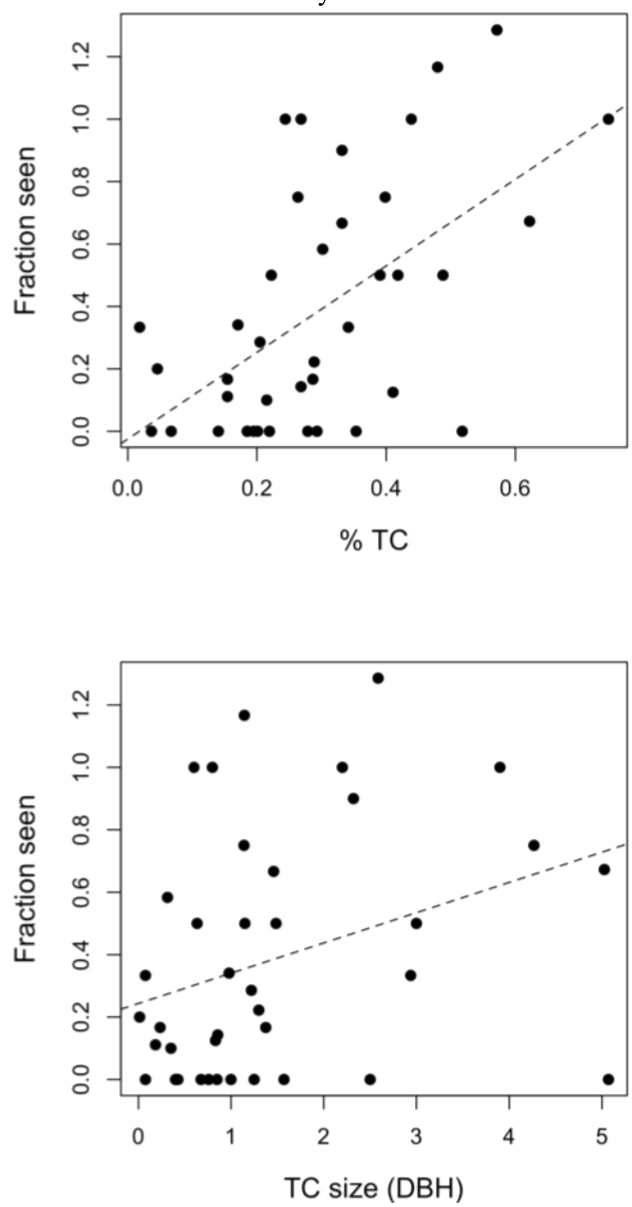



**Figure 6. Relationships between Tecate cypress attributes (density in the top panel and DBH in the bottom) and the fraction of survey points in which TH was observed per stand.**

includes a diversity of other woody and herbaceous plants as potential nectar sources.

3) Finally we note that our study points towards issues of spatial ecology as important for the conservation of butterflies. What configuration of host patches would promote optimal movement and resource location on a landscape-scale? This would not be easily addressed in TH, given the limited geographic range. However, closely related taxa living in similar habitats could be amenable to study and even manipulation.

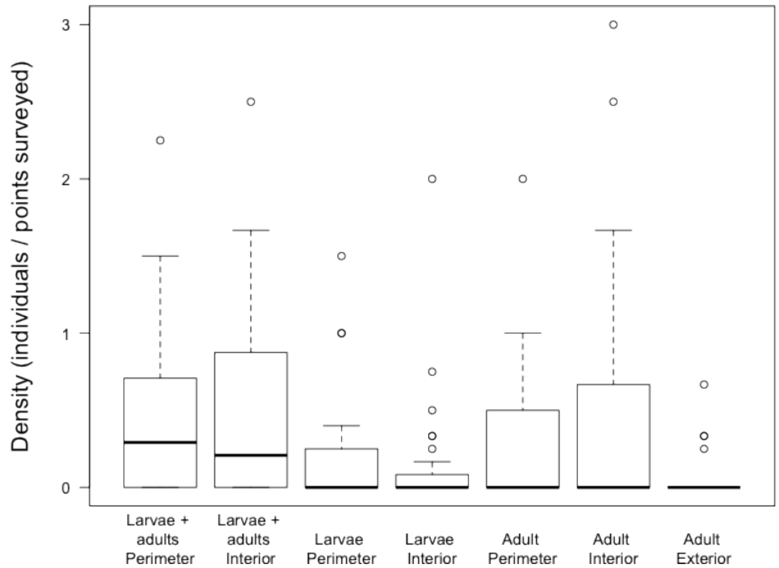



**Figure 7. Density of individuals observed in perimeter, interior and exterior survey points. Density here refers to the number of individuals observed per stand in the different spatial categories divided by the number of survey points in the same spatial categories.**

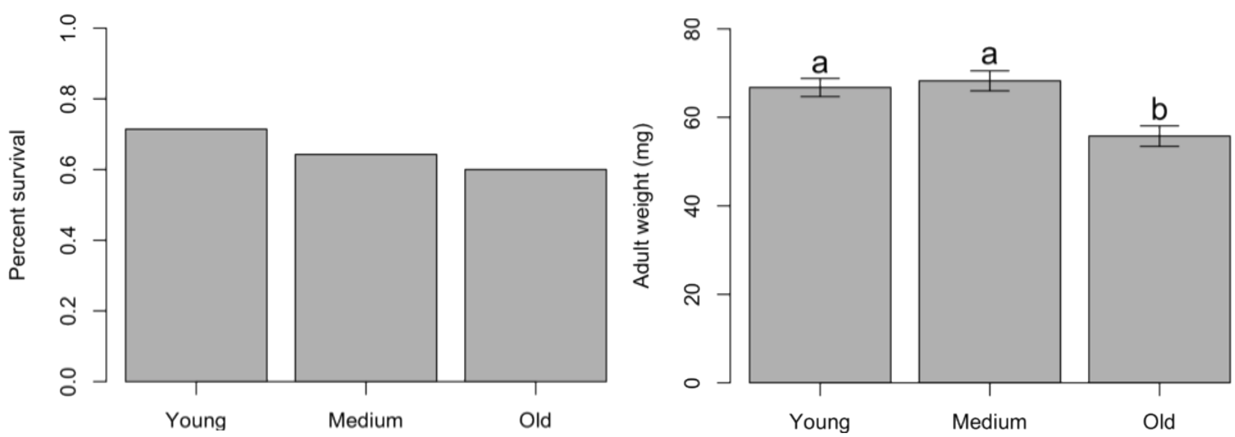



**Figure 8. Summary of results from *M. thornei* larval rearing experiment. Left panel shows survival of caterpillars reared on foliage from trees in three different age classes (no significant differences in survival). Right panel shows weights (means and standard errors) of pupae reared from caterpillars on the same three types of foliage. Lower case letters indicate significant differences at $P$ = 0.05.**

Appendix 1: Summary by stand, including UTM coordinates totals for plots surveyed.

| Stand # | UTM coordinates (N,E) | Acres | Total plots mapped | Plots surveyed for vegetation | Percent surveyed for vegetation | Plots surveyed for *M. thornei* | Total adult *M. thornei* observed |
|---|---|---|---|---|---|---|---|
| 1 | 512297.8, 3604681.3 | 10 | 18 | 11 | 61.11 | 10 | 5 |
| 3 | 512442.6, 3604294.8 | 51.4 | 27 | 17 | 62.96 | 2 | 9 |
| 5 | 511926.7, 3604594.8 | 0.2 | 6 | 2 | 33.33 | 0 | 0 |
| 6 | 511798.9, 3604567.7 | 8.7 | 6 | 6 | 100 | 4 | 2 |
| 7 | 511683.9, 3604314.1 | 8.7 | 18 | 9 | 50 | 1 | 0 |
| 8 | 511449.1, 3603856.8 | 10.2 | 21 | 13 | 61.9 | 3 | 5 |
| 9 | 511266.1, 3604109.2 | 16 | 33 | 33 | 100 | 12 | 3 |
| 10 | 509891.9, 3604392.0 | 2.6 | 9 | 3 | 33.33 | 3 | 0 |
| 12 | 510158.4, 3607926.9 | 33.2 | 30 | 15 | 50 | 11 | 3 |
| 13 | 514561.7, 3606428.4 | 90.3 | 48 | 12 | 25 | 13 | 0 |
| 14 | 517380.9, 3607354.0 | 3.8 | 12 | 12 | 100 | 9 | 0 |
| 15 | 513130.7, 3608721.5 | 37.5 | 33 | 24 | 72.72 | 10 | 9 |
| 16 | 514581.7, 3609694.6 | 6.2 | 15 | 11 | 73.33 | 10 | 0 |
| 17 | 513843.8, 3609704.1 | 0.4 | 6 | 6 | 100 | 6 | 1 |
| 18 | 514040.2, 3609683.2 | 1.7 | 9 | 9 | 100 | 7 | 0 |
| 19 | 514172.3, 3609790.4 | 2.5 | 9 | 9 | 100 | 6 | 2 |
| 20 | 514482.5, 3609896.6 | 0.3 | 3 | 2 | 66.66 | 3 | 0 |
| 21 | 510809.7, 3604241.6 | 31.2 | 24 | 8 | 33.33 | 4 | 3 |
| 22 | 509942.5, 3604623.5 | 17.1 | 18 | 8 | 44.44 | 6 | 1 |
| 23 | 510310.1, 3604500.7 | 19.1 | 24 | 11 | 45.83 | 8 | 1 |
| 24 | 511507.3, 3605013.8 | 41.2 | 39 | 8 | 20.51 | 7 | 2 |
| 25 | 510819.3, 3605315.0 | 36.6 | 36 | 10 | 27.77 | 7 | 0 |
| 26 | 512648.9, 3606147.5 | 16.1 | 18 | 12 | 66.66 | 3 | 0 |
| 27 | 511621.0, 3604035.7 | 5.1 | 15 | 15 | 100 | 11 | 2 |
| 28 | 512531.6 3603769.2 | 0.2 | 3 | 3 | 100 | 3 | 0 |
| 29 | 512654.6, 3603855.6 | 0.1 | 3 | 2 | 66.67 | 2 | 0 |
| 30 | 512684.7, 3603614.9 | 4.6 | 9 | 9 | 100 | 8 | 2 |
| 31 | 513368.3, 3604539.2 | 1.6 | 9 | 9 | 100 | 9 | 1 |
| 32 | 514816.9, 3608824.9 | 1.1 | 6 | 6 | 100 | 3 | 0 |
| 33 | 512519.1, 3609086.8 | 4.6 | 9 | 9 | 100 | 6 | 5 |
| 34 | 509838.9, 3605546.1 | 0 (linear) | 4 | 4 | 100 | 4 | 0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 35 | 510218.2, 3605178.9 | 1 (linear) | 4 | 4 | 100 | 4 | 0 |
| 36 | 510227.7, 3604849.1 | 2 (linear) | 12 | 7 | 58.33 | 5 | 8 |
| 37 | 511903.5, 3605008.8 | 3 (linear) | 4 | 2 | 50 | 1 | 0 |
| 38 | 514397.3, 3609972.3 | 4 (linear) | 2 | 1 | 50 | 2 | 1 |
| 39 | 514208.4, 3609720.1 | 5 (linear) | 2 | 2 | 100 | 2 | 0 |
| 43 | 512226.0, 3604660.4 | 9 (linear) | 4 | 2 | 50 | 2 | 3 |
| 45 | 509705.2, 3605168.9 | 11 (linear) | 2 | 2 | 100 | 2 | 0 |
| 46 | 509616.9, 3604836.3 | 13.2 | 18 | 18 | 100 | 8 | 2 |
| 47 | 509305.2, 3604820.2 | 1.3 | 9 | 9 | 100 | 9 | 5 |
| 48 | 509715.0, 3605054.2 | 0.2 | 3 | 3 | 100 | 3 | 0 |
| | | Average: 13.76 | Total: 624 | Total: 360 | Average: 73.27 | Total: 229 | Total: 75 |