University of Nevada, Reno

Thorne's Hairstreak (*Callophrys [Mitoura] thornei*) Monitoring
Third annual report, covering 2011
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Abstract:

This report covers activities performed during our third year of work with Thorne's hairstreak (TH). Activities are summarized with reference to objectives as outlined in our second annual report, and activities for 2013 are projected (the project will come to an end mid-2013). During the 2011 field season, we completed our surveys for vegetation and TH abundance. Over the course of both of our primary field seasons for this project (in 2010 and 2011), we surveyed a total of 358 plots for vegetation and 255 plots for TH presence and abundance associated with 40 stands of Tecate cypress. A total of 75 TH adults were observed in approximately half of the stands spread throughout the Otay Mountain study area. We also report here on results from habitat modeling, which suggests that host plant variables (e.g. cypress density and tree diameter) are statistically significant predictors of TH presence (while the density and richness of some potential nectar sources, for example, are not). These models, however, explain relatively little of the variation in TH observations, which we interpret in light of TH presence throughout the study area. In addition to observational studies, we were successful during the 2011 field season in carrying out experiments with TH larvae: 86 larvae were exposed to Tecate cypress foliage collected from trees that were either young, of medium age, or relatively old. Contrary to previous reports involving TH, but consistent with other work in the genus *Mitoura*, younger foliage was a superior larval resource. Future work would be needed to draw inferences regarding consequences for adult fitness in the wild. In summary, most tasks have been completed, and most objectives have been met for this project, including the experiment with caterpillars, which was previously uncertain given the sporadic availability of females.

Objectives for 2011 (Tasks 4, 5, and 6, see MOU):

(1) Complete occupancy surveys for TH.
(2) Complete vegetation work for previously unsurveyed plots.
(3) Age trees by coring and counting rings in cores.
(4) Conduct larval experiments to assess the importance of tree age for TH.
(5) Analyze data from 2010 and 2011 and prepare annual report.

Objectives for 2012 (Tasks 4, 5, and 6):

(6) Finish counting rings in tree cores.
(7) Complete final analyses involving habitat models for TH.
(8) Prepare a publication for an ecological journal on TH habitat requirements and conservation.
(9) Prepare a final report summarizing all analyses and implications for TH management.
(10) Present results to the SANDAG Environmental Program Working Group.

Description of completed activities:

Vegetation mapping and TH surveys

Vegetation work was carried out in 2011 as a continuation of activities described in our previous annual reports. Specifically, our survey units were 10 meter diameter plots randomly located in and around stands of Tecate cypress (stands were located and mapped by us in 2009). Within plots, a number of measurements were taken, including physical characteristics such as slope and temperature, and biotic characteristic such as the abundance and identity of possible nectar sources and the abundance and density of Tecate cypress (within each plot, vegetation data was taken at 41 points, along 4 transects running out from the center of a plot). In conducting vegetation surveys, the crew (2 people in the field) moved around the mountain by randomly picking a cypress stand each field day, and then a random set of plots within a stand (spreading energy equally among interior, edge and adjacent/exterior plots). As stated in our previous annual reports, these vegetation surveys follow the procedures outlined in the San Diego Multiple Species Conservation Plan, though we modified them to suit the logistical constraints of a 2-person field crew. Details on the number and location of stands surveyed can be seen in Table 1. All maps of the study location, including plots surveyed, are housed at the USFWS offices in Carlsbad, with backups in Reno, Nevada. We are currently working on high-quality maps that will accompany our final report for the project and a publication.

Surveys for TH were conducted both for adults (butterflies) and juveniles (caterpillars). By targeting both of these life history stages, we were able to increase the number of survey days: adults and larvae were observed during the adult flight periods, and larvae only were surveyed other times, primarily in the weeks intervening between the two main broods. By adding larval surveys to our plan, we also gained valuable information because the presence of larvae increases the probability that a given tree and stand supports a population (as opposed to the observation of an adult, which could always be an ephemeral individual moving through and area). Adult surveys were conducted by searching within plots for 5 minutes, and noting the activity and position of TH individuals when detected (searching included tapping vegetation with a pole). When possible, the identity of butterflies was confirmed by brief capture in a net. Larval surveys were conducted using "beat sheets" under randomly selected trees within plots (8 total trees per plot). Details on the number and location of plots in which TH were observed can be seen in Table 1. Note that we only report here data on TH adults; caterpillar observations are still being synthesized and analyzed, and will be included in our final report.

As we began our 2011 field season, we had to make a choice of whether to resample plots for TH that had been studied in the previous year, sample only new plots, or a combination of both. With a sufficiently large field crew, the ideal strategy would have been the combination, which would have allowed us to address temporal consistency (comparing TH presence between years) and discover new locations containing TH. Considering the size of our field crew (2 people), the combined strategy would have left us with insufficient data for a temporal comparison and insufficient opportunities to discover new locations. Thus we took the strategy in 2011 of focusing on plots that had not been surveyed for TH in 2010. We believe that this choice has been justified by our results: TH has been discovered from a large number of stands, which is perhaps our most important finding for conservation and management. However, the sampling strategy in 2011 should be kept in mind when interpreting statistical results from habitat modeling, which could not partition variation between years (see below).

Analyses: habitat modeling

All data has been collected and analyzed to a certain extent: we have taken a first pass at habitat modeling for adult occurrences, and will continue to refine these results over the coming months. While we believe that the results reported here will likely stand in the face of future analyses, these results should still be considered preliminary until our final report has been submitted at the conclusion of the project.

Our final report will also contain a more detailed description of statistical methods, but, in brief, our analyses have consisted of the following steps: (1) we create “environmental” matrices based on the abiotic and biotic variables (not including TH abundance) observed in our plots; (2) these environmental matrices are converted to triangular distance matrices (in which a single value expresses how similar or dissimilar two plots are based on the observed variables); (3) a matrix of TH observations is used to create a triangular distance matrix; (4) permuted (Mantel) analyses (appropriate for distance matrices) are used to address correlations between the environmental variables, the TH matrix, and a geographic distance matrix (meters between plots, which allows us to control for spatial autocorrelation). These analyses were done both with all plots, and focusing only on plots in which TH was observed (for the former, the data consisted only of presence and absence, while for the latter, the data consisted of abundances). Thus analyses can be thought of as addressing two questions: (1) where is TH found with respect to environmental variables? and (2) of the places where TH is found, which environmental variables predict abundance?

Mantel tests were performed in three ways. First, we conducted pairwise comparisons between TH and each environmental variable. Second, we focused only on variables significant in the pairwise comparisons, and included them in partial Mantel tests where each variable was again compared to TH, but this time accounting for geographic distance (potential spatial autocorrelation). Finally, a multiple partial Mantel test was run to understand the potential influence of multiple variables while accounting for the possibility that the variables are themselves correlated and potentially explain the same variation in TH presence and abundance.

Results suggest that some of our observed variables are significant predictors of TH occurrence, as can be seen in Table 2. For example, Tecate cypress size (DBH), density, and crown density are all significant predictors of adult TH presence. It is interesting to note that some variables potentially encompassing nectar sources (woody richness and herbaceous richness) are not significantly associated with TH presence. Despite the significance of the observed relationships reported in the first rows of Table 2, a more complete model involving multiple variables (see the last rows of Table 2) reveals relatively little variation explained: $R^2 = 0.029$ ($F = 136.13$, $P = 0.001$). We interpret this last result as reflecting the fact that TH was documented in plots throughout the study area, encompassing much of the environmental variation in the area. A second set of analyses were done utilizing only plots in which TH was observed, but this time using abundance data instead of presence/absence. These analyses were not able to discriminate among plots based on environmental variables (Table 3).

Three important methodological caveats can be offered at this time. First, we are still working on similar data for caterpillar occurrence, which will provide an interesting counterpoint to results from adults, and might reveal different associations with environmental variables.

Second, we have not yet done similar analyses at the stand level (current analyses are at the plot level). Finally, the analyses of presence/absence matrices (as in Table 2) involve a great many "ties" – these are nonparametric analyses, and similar values (i.e. many 0s) are given the same rank. Although not directly violating any assumption of the rank-based correlations, statistical advice is usually to avoid "too many" ties. However, we believe that the analyses we have done still have heuristic value, because they are simple correlations that can be readily understood, and they ultimately will be paired with other analyses (in particular at the stand level) that will not be dominated by ties.

Analyses: larval experiment

One of the goals of our project has been to address the possibility that TH caterpillars do not perform equally well when consuming Tecate cypress foliage of different ages. An original hypothesis, based on observations of lepidopterists, was that TH primarily associated with more mature trees, raising the possibility that survival was somehow compromised in stands lacking mature trees. Subsequent to that observation, results presented by another research group suggested that young foliage was not inferior for caterpillars, though these conclusions did not involve caterpillars reared to adults. We obtained 86 larvae from wild-caught TH females, and reared 56 of them to adults on foliage of three different ages. Larvae were reared individually in large petri dishes, as we have done with other butterflies in the same genus and many other species of butterfly in the same family (Lycaenidae). Rearing was done at the University of Nevada, Reno, under ambient room-temperature conditions and 12hr/12hr light/dark cycles.

Foliage for experiments was collected from a single spot on Otay Mountain that was chosen because it had a diversity of Tecate cypress age classes in immediate proximity. The three different ages of trees were represented by three size classes: trees less than 2 meters in height, between 2 and 3 meters, and greater than 3 meters. From each size (age) class, fresh foliage was selected, and different trees were sampled on repeat collection visits.

We did not detect a difference in survival on the three types of foliage (Chi-square = 0.18, P = 0.92), as can be seen in the top panel of Fig. 1. However, we did detect a highly significant effect of foliage age on adult weight: individuals reared on the older foliage eclosed as smaller adults, as can be seen in the bottom panel of Fig. 1 ($F_{2,53} = 9.35$, P = 0.00033). Although the absolute effect was small, we can expect that variation in adult size is correlated with adult fecundity, as has been observed in other butterflies and other lycaenids in particular. We do not, of course, know if any effect on adult fecundity would translate to meaningful differences in population growth in the field. However, we can at least confirm that older foliage is not a superior larval resource for TH: if anything, it is slightly inferior. These results should be considered with an important caveat: more mature trees could be quite important for TH populations because they could serve as locations for lekking behavior, where males congregate to find and attract mates. That possibility (an effect on lekking) is one that we considered in our original proposal to work with TH. We suggested the possibility of manipulating tree size and observing TH responses in the field. Given the densities that we have observed in the field in our time on Otay Mountain, we now believe that this experimental approach with adult behavior would never be feasible in the wild. Thus it will likely always be impossible to rule out the possibility that mature trees are important to TH populations through their effect on adult behavior, but not necessarily on larval performance.

Challenges in 2011:

Age trees

The objective of aging Tecate cypress stands by taking and counting tree cores has been a persistent challenge for us. In our first full summer, we did not have the best preservation technique, and most cores did not survive transportation from the field. In the second full summer (of 2011), we used a different preservation method which allowed us to bring most cores back to UNR. These have not been processed, as we have prioritized data entry and analysis. However, we will count rings on tree cores in the remaining months of the project. We will focus these efforts on cores from stands which can not be aged from fire maps.

Overview of activities projected in 2011:

Activities projected for the remainder of the project include finalization of habitat modeling analyses. We will continue to develop models at the plot level (where plots are our individual sampling points in and around cypress stands). We will also conduct analyses at the stand level, involving summary statistics generated from plots within stands. Stand-level analyses provide an important counterpoint to interpretations at the more fine scale. In addition, stand-level analyses potentially provide more direct conclusions for management, targeting certain stands or types of stands as being more likely to support TH populations into the future. We will also complete all of the same analyses using presence and abundance of caterpillars, and we will also count tree rings as discussed above.

Table 1. Summary of survey activities associated with individual Tecate cypress stands. “Total plots mapped” refers to the number of GPS points that were randomly generated in association with each stand. Other columns report the number (and percentage for vegetation) of each of those plots that were surveyed for vegetation and Thornes' hairstreak (only adults) occurrence in 2010 and 2011.

Stand #	UTM coordinates (N,E)	Acres	Total plots mapped	Plots surveyed for vegetation	Percent surveyed for vegetation	Plots surveyed for TH	Total TH observed
1	512297.8, 3604681.3	10	18	11	61.11	11	5
3	512442.6, 3604294.8	51.4	27	17	62.96	1	9
5	511926.7, 3604594.8	0.2	6	2	33.33	0	0
6	511798.9, 3604567.7	8.7	6	6	100.00	4	2
7	511683.9, 3604314.1	8.7	18	9	50.00	1	0
8	511449.1, 3603856.8	10.2	21	13	61.90	6	5
9	511266.1, 3604109.2	16	33	33	100.00	12	3
10	509891.9, 3604392.0	2.6	9	3	33.33	6	0
12	510158.4, 3607926.9	33.2	30	15	50.00	12	3
13	514561.7, 3606428.4	90.3	48	12	25.00	13	0
14	517380.9, 3607354.0	3.8	12	12	100.00	9	0
15	513130.7, 3608721.5	37.5	33	24	72.72	11	9
16	514581.7, 3609694.6	6.2	15	11	73.33	9	0
17	513843.8, 3609704.1	0.4	6	6	100.00	6	1
18	514040.2, 3609683.2	1.7	9	9	100.00	10	0
19	514172.3, 3609790.4	2.5	9	9	100.00	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	7	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	6	0
26	512648.9, 3606147.5	16.1	18	12	66.66	7	0
27	511621.0, 3604035.7	5.1	15	15	100.00	11	2
28	512531.6 3603769.2	0.2	3	3	100.00	3	0
29	512654.6, 3603855.6	0.1	3	2	66.67	3	0
30	512684.7, 3603614.9	4.6	9	9	100.00	10	2
31	513368.3, 3604539.2	1.6	9	9	100.00	9	1
32	514816.9, 3608824.9	1.1	6	6	100.00	6	0
33	512519.1, 3609086.8	4.6	9	9	100.00	8	5
34	509838.9, 3605546.1	0 (linear)	4	4	100.00	6	0
35	510218.2, 3605178.9	1 (linear)	4	4	100.00	6	0
36	510227.7, 3604849.1	2 (linear)	12	7	58.33	5	8
37	511903.5, 3605008.8	3 (linear)	4	2	50.00	1	0
38	514397.3, 3609972.3	4 (linear)	2	1	50.00	1	1
39	514208.4, 3609720.1	5 (linear)	2	2	100.00	3	0
43	512226.0, 3604660.4	9 (linear)	4	2	50.00	2	3
45	509705.2, 3605168.9	11 (linear)	2	2	100.00	2	0
46	509616.9, 3604836.3	13.2	18	18	100.00	7	2
47	509305.2, 3604820.2	1.3	9	9	100.00	10	5
48	509715.0, 3605054.2	0.2	3	3	100.00	3	0
		Average: 12.48	Total: 624	Total: 360	Average: 73.27	Total: 255	Total: 75

Table 2. Results from Mantel, partial Mantel, and multiple partial Mantel analyses with environmental variables and TH adult presences/absences. These are comparisons among distances matrices for each variable; Mantel test are permuted, which is appropriate for the non-independence of data within distance matrices. These analyses were done at the plot level, with all data (including plots where TH was observed and all plots in which TH was never observed). Within each type of test below, variables are listed in order of the magnitude of their correlation with TH presence, from most positive to most negative. The variables included in the second two sets of analyses (the partial Mantel tests and the multiple Mantel tests) were the variables that were significant in the pairwise tests.

	Variable	Correlation coefficient	P
Mantel	Average Tecate cypress DBH (diameter at breast height)	0.134	0.001
	Tecate cypress density	0.118	0.001
	Tecate cypress crown density	0.091	0.006
	Average Tecate cypress height	0.067	0.005
	Slope	0.054	0.009
	Density of woody plants, excluding Tecate cypress	0.034	0.044
	Woody Richness, excluding Tecate cypress	0.011	0.342
	Size of stand (nearest to plot)	0.008	0.357
	Leaf Litter, Rock, and Bare Ground	0.003	0.441
	Wind speed during observations	-0.001	0.472
	Herbaceous richness	-0.016	0.709
	Cloud cover during observations	-0.024	0.777
	Distance between plots	-0.023	0.803
	Temperature during observations	-0.037	0.989
	Herbaceous density	-0.06	0.993
Partial Mantel	Average Tecate cypress DBH (diameter at breast height)	0.132	0.001
	Tecate cypress density	0.12	0.001
	Tecate cypress crown density	0.088	0.006
	Average Tecate cypress height	0.066	0.005
	Slope	0.054	0.019
	Density of woody plants, excluding Tecate cypress	0.034	0.073
Multiple Mantel	Average Tecate cypress DBH (diameter at breast height)	0.12	0.003
	Tecate cypress density	0.071	0.002
	Slope	0.041	0.025
	Density of woody plants, excluding Tecate cypress	0.024	0.10
	Tecate cypress crown density	-0.0051	0.84
	Distance between plots	-0.025	0.14
	Average Tecate cypress height	-0.057	0.044

Table 3. Results from Mantel tests with environmental variables and TH abundance, based on distance matrices as in Table 2. Unlike Table 2, these analyses used abundance (rather than presence/absence) and focused only on plots in which TH was observed. In other words, these analyses addressed the question: for the locations in which TH exists, what explains variation in abundance? Further analyses (e.g. partial Mantel tests, as in Table 2) were not performed here because none of the pairwise relationships were significant. Variables are listed in order of the magnitude of their correlation with TH presence, from most positive to most negative.

Variable	Correlation coefficient	P
Cloud cover during observations	0.095	0.114
Temperature during observations	0.088	0.100
Size of stand (nearest to plot)	0.068	0.140
Tecate cypress crown density	0.06	0.116
Tecate cypress density	0.052	0.147
Herbaceous density	0.042	0.247
Average Tecate cypress DBH (diameter at breast height)	0.035	0.304
Wind speed during observations	0.025	0.247
Woody Richness, excluding Tecate cypress	0.023	0.289
Average Tecate cypress height	-0.005	0.492
Slope	-0.018	0.592
Herbaceous richness	-0.027	0.617
Density of woody plants, excluding Tecate cypress	-0.087	0.988
Leaf Litter, Rock, and Bare Ground	-0.103	0.915

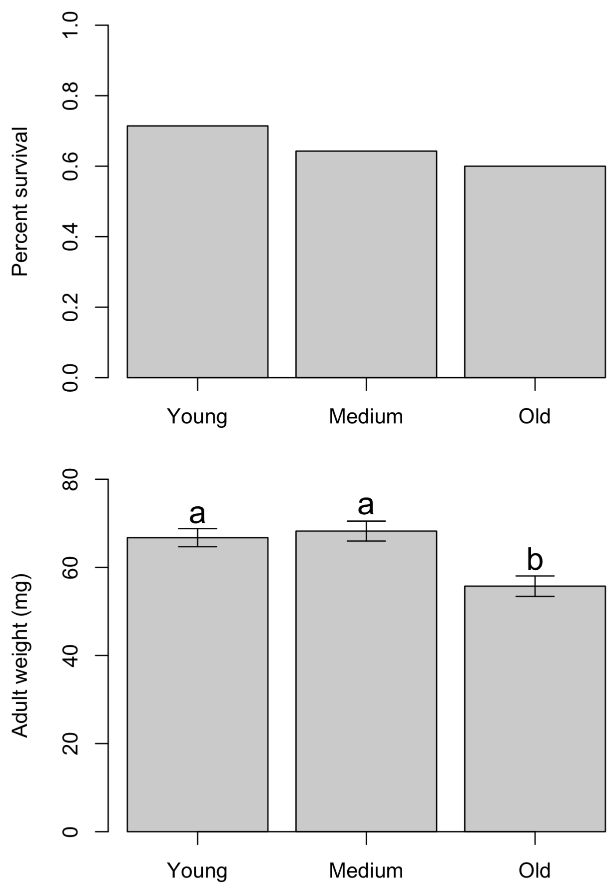

Fig. 1. Summary of results from TH rearing experiment. Top panel shows survival of caterpillars reared on foliage from trees in three different age classes (no significant differences in survival). Bottom panel shows weights (means and standard errors) of adult butterflies reared from caterpillars on the same three types of foliage. Lower case letters indicate significant differences at $P = 0.05$.