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**TWO-YEAR EVALUATION OF  
HERMES COPPER (*LYCAENA HERMES*)**

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**ON CONSERVED LANDS IN SAN DIEGO COUNTY**

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## INTRODUCTION

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The Hermes copper butterfly, *Lycaena [Hermelycaena] hermes* is a rare butterfly endemic to the coastal sage scrub (CSS) community in San Diego County and northern Baja California. Conservation groups and wildlife agencies recognize that Hermes copper is threatened by recent urbanization and wildfires. Until recently gaps in knowledge about Hermes copper prevented its listing as threatened or endangered (see 2010 final report for discussion of previous research and status reviews). This project was initiated in 2010 in order to evaluate the size and distribution of Hermes copper populations in San Diego County. In early 2011, Hermes copper was added to the candidate species list by USFWS (US Fish and Wildlife Service 2011). It now awaits the development of a proposed rule before being formally listed.

### GOALS AND OBJECTIVES

The overall goal of this project is to minimize the risk that Hermes copper will become extinct. To reach this goal, we must meet the following initial objectives:

- (1) improve our basic understanding of population status and trend
- (2) describe natural and anthropogenic threats to the species
- (3) evaluate potential management options to ameliorate threats and/or to increase the size and range of viable populations

In the first year of this project, we provided an initial evaluation of Hermes copper populations on conserved land in San Diego County. In 2011, we continued surveying many of the same sites to further assess the distribution and document fluctuations in population size. In addition some new sites were surveyed. This second year of the project was organized around three individual tasks, each a critical part of understanding the status of Hermes copper in San Diego (Table 1).

**Table 1:** Project goals and objectives from 2010 and 2011.

<b>Task 1: Field Surveys</b>
Survey the locations established in 2010 to investigate population fluctuations Survey new sites and/or historical locations Evaluate sites for evidence of post-fire recolonization
<b>Task 2: Landscape Genetics</b>
Evaluate non-lethal sampling technique (mark/recapture study) Evaluate dispersal ability Process 2010 specimens using AFLP
<b>Task 3: Data Analysis and Synthesis</b>
Synthesize and analyze this year's data Report on current range and size of Hermes copper populations in San Diego Compare 2010 and 2011 field seasons Study population structure, behavior and survey methods Identify critical uncertainties about the species.

The primary objective **Task 1** was to document the presence and estimate relative population size of Hermes copper at as many sites in San Diego as possible. Last year we identified several new populations and quantified the population at those sites. One key goal this year was to determine if the population sizes were consistent from year to year. We identified new sites to visit based on the presence of spiny redberry (*Rhamnus crocea*), fire history and historic occupancy. In addition we continued to survey sites which were unoccupied in 2010 in order to confirm absence.

Although some of the historical sites we visited were in areas burned in 2003 and/or 2007, we did not have time to check all previously identified populations of Hermes or redberry inside the fire perimeters. Although evaluating if populations survived the fires is an important question, it was not our primary focus. Data collected after the fires suggests that re-colonization is extremely rare, even when adequate redberry is present (Marschalek and Klein 2010). Since our primary focus was to quantify population size and temporal change, we allocated most of our field effort to the sites which were occupied in 2010.

**Task 2** represents a very different approach to understanding the status of Hermes copper. We analyzed genetic material collected during the 2010 field season. We used amplified fragment length polymorphism (AFLP) to characterize the genetic differences among individuals both within and among different sites. These data on genetic differences allowed us to draw inferences about previous dispersal events and genetic differentiation within the species.

**Task 3** is a comprehensive analysis of the field and genetics data collected in 2010 and 2011. The analysis provided an opportunity to compare survey methods and to propose new or revised methods. Ultimately, this task culminated in an initial conceptual model for monitoring and management of Hermes copper.

This report is organized around the major tasks of the 2011 project. For each task, we present information on our methods, summarize the results, and discuss their relevance. In addition, we list

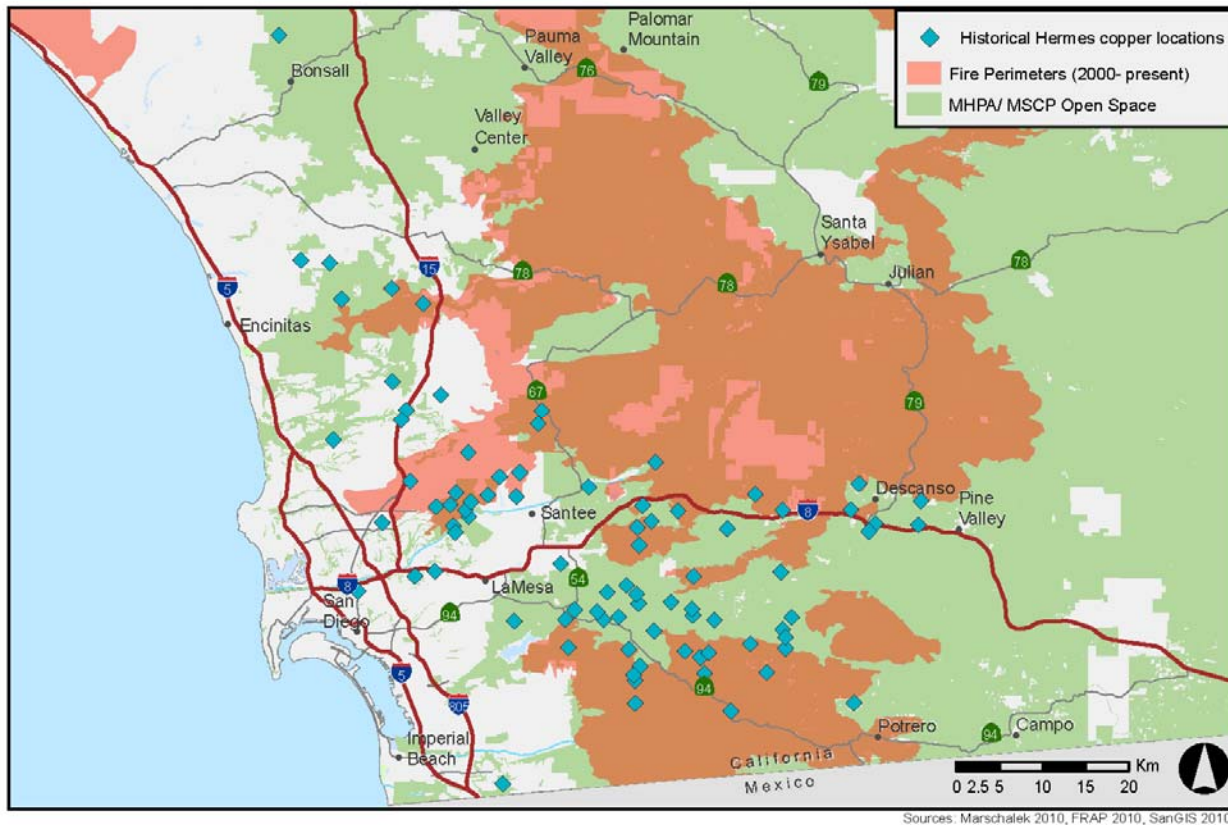


critical uncertainties as part of the adaptive management framework used by agencies and land managers in San Diego County.

## **BIOLOGY AND LIFE HISTORY OF HERMES COPPER**

In the United States, Hermes copper is only found within San Diego County, west of the Cuyamaca Mountains (Thorne 1963; Brown 1991; Faulkner and Klein 2004; Marschalek 2004; see Map 1). They also occur in northern Baja California, Mexico, however very little is known about the status of the butterfly south of the United States-Mexico border (Thorne 1963; Emmel and Emmel 1973; Marschalek and Klein 2010). They have been recorded as far north as the community of Fallbrook, in San Diego County and as far south as Ensenada in Mexico. They have never been recorded immediately along the Pacific coast, and have not been found further east than the western slopes of the mountains above 1300 meters (Marschalek and Klein 2010).

Hermes emerge in the late spring after overwintering as eggs and spend a short period of time as caterpillars (Thorne 1963; Faulkner and Klein 2004). Adult emergence is fairly consistent, generally beginning in mid- to late May, with the flight period extending to between late June and mid-July (Faulkner and Klein 2004; Marschalek and Deutschman 2008; Marschalek and Klein 2010). Emergence appears to be influenced by climactic conditions; however our understanding of this relationship is incomplete. For example, 2010 was cool and moist and the Hermes flight season was delayed. In contrast, 2006 was hot and dry and also had a late emergence period (Marschalek and Klein 2010). More comprehensive data are needed to understand this relationship. Virtually nothing conclusive is known about the ability of eggs and larvae to undergo diapause during years with poor conditions.



Sources: Marschalek 2010, FRAP 2010, SanGIS 2010

**Map 1:** Historical range of Hermes copper. Adapted from Marschalek and Klein (2010). Sites are denoted with a blue diamond. Public conserved lands are shaded in green. The 2003 and 2007 wildfires are shaded in orange and pink.

Hermes larvae use only spiny redberry as a host plant (Thorne 1963; Brown 1991; Faulkner and Klein 2004). Eggs are laid, typically, at the intersection of branches on new growth (Marschalek and Deutschman 2009). Although adults nectar almost exclusively on California buckwheat (*Eriogonum fasciculatum*) they are rarely found far from a spiny redberry plant (Thorne 1963; Brown 1991; Faulkner and Klein 2004; Marschalek 2004). A more detailed understanding of suitable habitat is lacking. For example, it is not clear how much redberry and/or buckwheat is necessary to support a Hermes copper population in a given area.

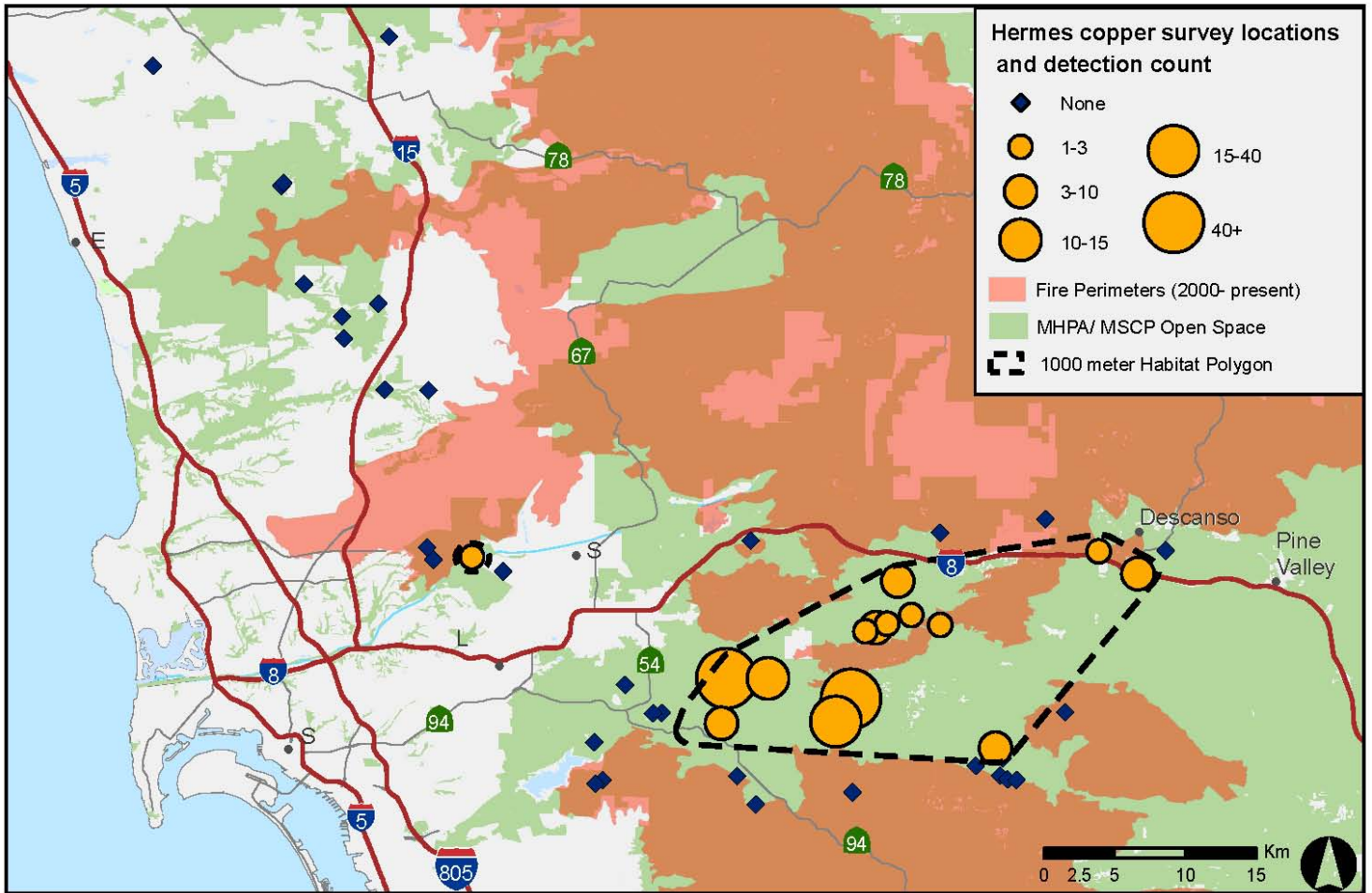
During the flight season, Hermes copper adults become active at around 22°C (72°F) (Marschalek 2004; Marschalek and Deutschman 2008). Adult males have a strong preference for openings in the vegetation, including roads and trails, specifically for the north and west sides of openings (Marschalek 2004; Marschalek and Deutschman 2008). Likewise they prefer to perch on the south and east sides of shrubs (Marschalek 2004; Marschalek and Deutschman 2008). They tend to remain inactive or sluggish under conditions of heavy cloud cover and cooler weather (Marschalek 2004; Marschalek and Deutschman 2008).

Hermes copper typically exhibit short movements with the majority of their movements well under 50 meters (Marschalek 2004; Marschalek and Klein 2010). Movements only rarely exceed 100 meters, and

the longest movement reported for a Hermes copper is just over 1 kilometer (Marschalek 2004; Marschalek and Klein 2010).

### **SUMMARY OF 2010 RESULTS**

Last year we identified 42 unburned sites with at least some redberry shrubs inside the range of Hermes copper in San Diego (see 2010 final report for more information). Of those we prioritized 33 for surveys, and found that 13 sites were occupied (Map 2). Of those 13 sites 5 were previously unreported populations. Over the course of 136 site visits we counted a total of 183 Hermes copper across San Diego County. In addition, four sites surveyed by other biologists were found to be occupied. All of the occupied sites (save for one) occur in a small section of unburned land in the southeast of the county, from Descanso in the North East to Jamul in the south west (Map 2). This section represents about 2.7% of the land area in the county, or about 10,878km<sup>2</sup>.



**Map 2:** Detections of Hermes copper butterflies on conserved lands, 2010. Black diamonds mark sites with no detections. Orange circles represent sites with Hermes copper. Circle size is proportional to the total number of Hermes copper butterflies recorded (Pollard Index). The dashed polygon encloses all but one of our documented populations using a 1000m buffer around all points. The lone outlier was the detection at Mission Trails Regional Park.

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## TASK 1 – *FIELD SURVEYS*

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### IDENTIFYING POTENTIAL NEW SITES

After the 2010 flight season, we compiled a list of sites that could potentially have suitable habitat. Candidate sites were identified based on discussions with USFWS. These candidate sites were checked for the presence of spiny redberry before the 2011 flight season, and if present were surveyed in 2011 (See Table 2). In addition we checked Lake Jennings during the peak of the flight season, which was known to have some redberry shrubs but was not surveyed in 2010.

**Table 2:** New sites checked for spiny redberry.

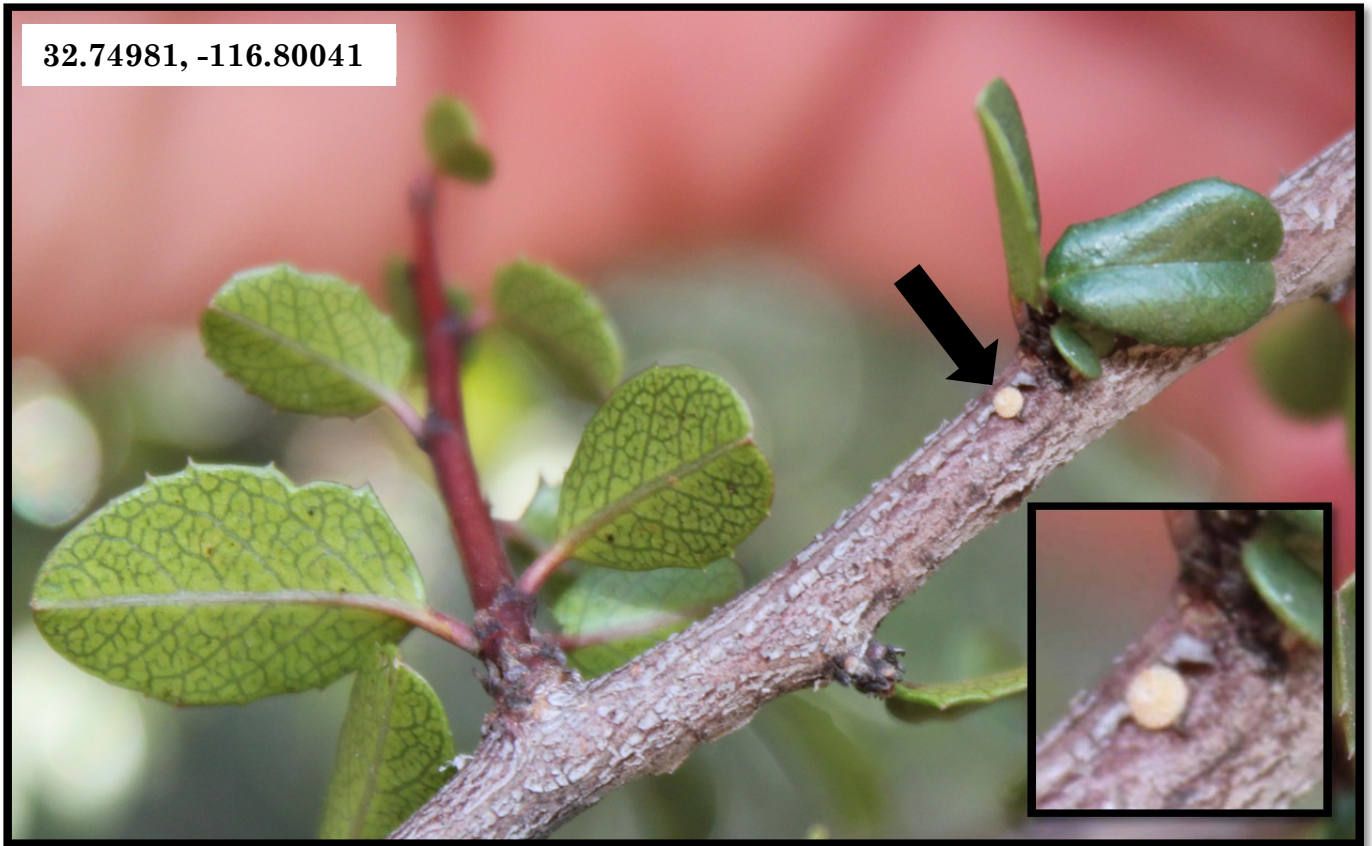
Site	Result
Sandia Creek	No Redberry
Daley Ranch	No Redberry
Dixon Lake	No Redberry
Lake Wholford	No Redberry
Dictionary Hill	Few redberry, damaged habitat
Medocino	Redberry
Lopez Canyon	Redberry

### SEARCHING FOR EGGS

Female butterflies deposit single, white, semi-spherical eggs on spiny redberry (Marschalek and Deutschman 2009). Hermes copper eggs are approximately one millimeter in diameter. Although the surface detail on each egg is very distinctive, their small size and isolation make them extremely difficult to find in the field. In a laboratory setting, females generally choose to lay eggs on or near new growth, at a branch intersection or under a leaf (Marschalek and Deutschman 2009).

In January of 2010, we conducted limited searches for Hermes copper eggs at Sycuan Peak (the most densely populated area in 2010). Over the course of several hours of work a single egg was located and identified midway up Sycuan Peak (Figure 1). The egg was located near new growth, underneath a node with a cluster of leaves on a branch fairly low on the west side of the bush. Unfortunately the image is somewhat blurry when it is enlarged to show surface details, but the general shape is clear. These observations reflect the selections for egg positioning behavior observed in the lab (Marschalek and Deutschman 2009).

32.74981, -116.80041



**Figure 1:** Hermes copper egg located in January of 2011. Hermes tend to select sections of branch near new growth, and lay eggs at branch intersections or underneath leaves.

## TRAINING AND TESTING

In 2010, we developed a rigorous program for training and testing member of our field team. Team members were provided a list of ~50 butterfly species detected by Marschalek during butterfly surveys in previous years (Table 3). The team studied images, descriptions and pinned specimens. They were required to pass a test before becoming certified to conduct surveys independently (see 2010 final report for details about the training program and testing rules). Most of our field members were experienced surveyors who had worked for us in 2010. Even so, all returning members were retested along with the new members of the field crew.

**Table 3:** Common names of butterflies detected during previous studies. This list was compiled DA Marschalek.

American Lady	Hedge-Row Hairstreak
Behr's Metalmark	Hermes Copper
Bernardino or Dotted Blue	Lorquin's Admiral
Boisduval's Blue	Lupine or Acmon Blue
Brown Elfin	Marine Blue
Buckeye	Monarch
Cabbage White	Mt. Mahogany Hairstreak
California Dogface	Northern White Skipper
Comstock's Fritillary	Orange Sulphur
California Hairstreak	Painted Lady
California Ringlet	Pale Swallowtail
California Sister	Pygmy Blue
Checkered White	Queen
Cloudless Sulphur	Reakirt's Blue
Dainty Sulphur	Red Admiral
Edward's Blue	Rural Skipper
Fiery Skipper	Sara's Orangetip
Funeral Duskywing	Silver Spotted Skipper
Gabb's Checkerspot	Silvery Blue
Gray Hairstreak	Sleepy Orange
Great Copper	Sylvan Hairstreak
Great Purple Hairstreak	Tiger Swallowtail
Great Basin Wood-Nymph	West Coast Lady
Harford's Sulphur	White Checkered Skipper

## HERMES COPPER SURVEYS

We used Sycuan Peak as an indicator site based on the ease of access, the high population size in 2010, and the proximity to Skyline Truck Trail where previous research shows Hermes tend to emerge early. We began checking Sycuan Peak informally, once a week, starting the first week of May. In addition to being our monitoring trigger, Sycuan Peak was also the location of a mark-recapture study aimed at determining if non-lethal genetic sampling was possible. As a result Sycuan peak was usually surveyed 3 times a week, instead of the standard one visit per week used at all other sites. In order to account for the increased sampling, we use only the midweek survey for calculating the Pollard counts, our index of relative population size.

In 2011, the first Hermes copper adult was observed flying on May 31<sup>st</sup> at Sycuan Peak, and the last two were observed on July 6<sup>th</sup> at Roberts Ranch. In 2010, Hermes were detected between May 29<sup>th</sup> and July 2<sup>nd</sup>. The start of both flight seasons were later in the season than we anticipated based on recent observations (Marschalek and Klein 2010). Even so, these dates fall within the range of emergence periods described in the literature.

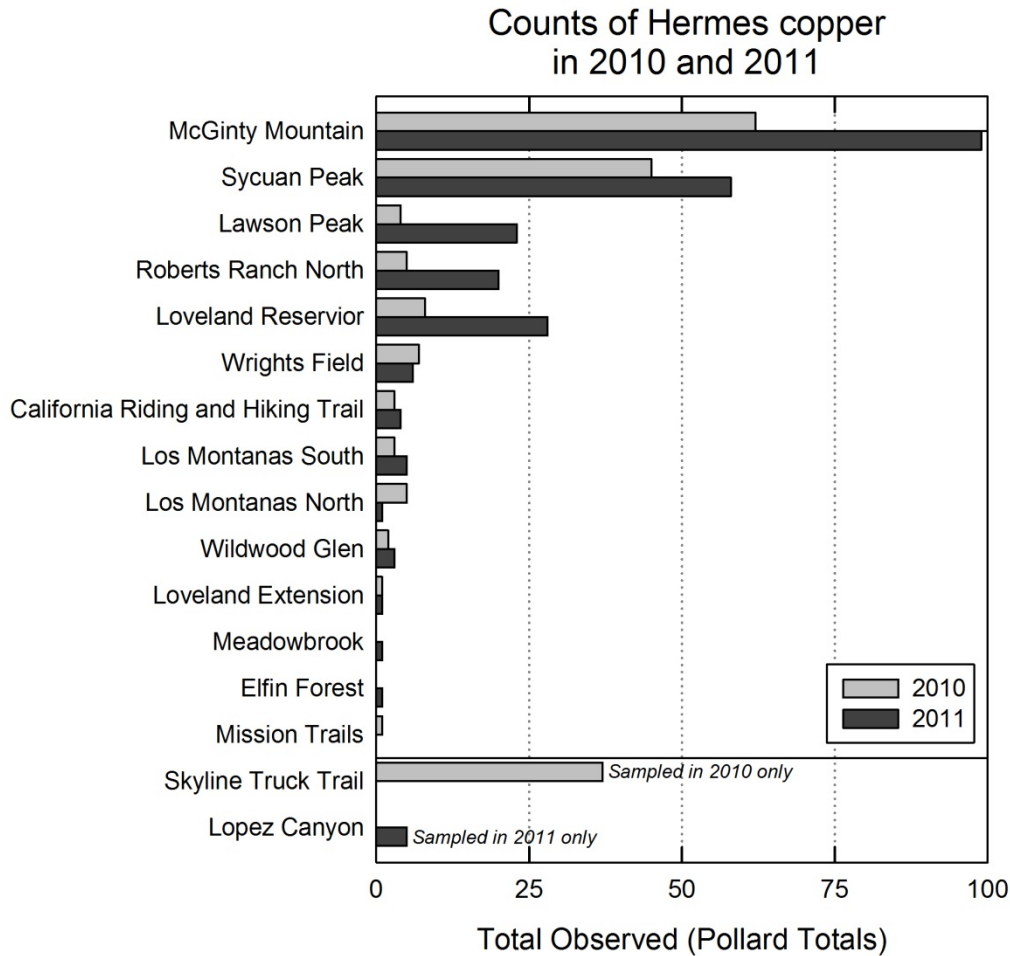
Beginning on May 31<sup>st</sup> teams began visiting 65 routes across 35 sites at the shortest interval possible, about once a week for most routes (Table 4). Our effort across sites was not homogenous, based on the priority of the site, the status of the buckwheat at the site, and how long the Hermes copper persisted if they were present. Top priority sites received a minimum of three visits between May 24<sup>th</sup> and July 11<sup>th</sup>.

**Table 4: Hermes copper survey locations and counts.** Note that some routes were added or modified in 2011. In addition, frequent visits to Sycuan Peak for the mark-recapture study are included as an extra line in the table and are not used in the calculation of the 2011 totals.

<b>Sites with at least 1 Hermes detected</b>				<b>2010</b>			<b>2011</b>		
Lat	Long	Notes	Visits	Pollard Total	Max Count	Visits	Pollard Total	Max Count	
32.755	-116.860	<i>Route modified</i>	7	62	26	10	99	27	
32.747	-116.800		9	45	12	5	58	27	
32.747	-116.800		.	.	.	17	98	27	
32.797	-116.772		5	8	3	5	28	10	
32.715	-116.706		4	4	2	5	23	15	
32.826	-116.616		4	5	4	7	20	9	
32.728	-116.899		4	3	1	4	5	3	
32.827	-116.767		3	7	4	5	6	3	
32.800	-116.762		4	3	2	5	4	2	
33.075	-117.159		3	0	0	3	2	1	
.	.	<i>New route</i>	.	.	.	2	5	5	
32.841	-116.632		5	2	1	6	3	2	
32.732	-116.894		4	5	3	4	1	1	
32.963	-117.069	<i>Route modified</i>	3	0	0	4	1	1	
32.834	-117.041		4	1	1	3	0	0	
32.790	-116.743		4	1	1	5	1	1	
32.732	-116.806	<i>Access Denied</i>	15	37	9	.	.	.	
<b>Sites with no detections</b>				<b>2010</b>			<b>2011</b>		
Lat	Long	Notes	Visits	Pollard Total	Max Count	Visits	Pollard Total	Max Count	
32.852	-116.743		2	0	0	2	0	0	
32.704	-116.719	<i>Route modified</i>	3	0	0	3	0	0	
32.944	-117.069		3	0	0	3	0	0	
32.977	-117.116		7	0	0	3	0	0	
32.827	-117.020		4	0	0	3	0	0	
32.823	-116.864		4	0	0	5	0	0	
32.757	-116.944		3	0	0	2	0	0	
33.149	-117.243		4	0	0	3	0	0	
.	.	<i>New</i>	.	.	.	2	0	0	
32.846	-116.861		2	0	0	2	0	0	
32.836	-116.596	<i>Route modified</i>	2	0	0	1	0	0	
32.695	-116.812		2	0	0	2	0	0	
33.168	-117.095		3	0	0	3	0	0	
33.003	-117.152	<i>Retired</i>	2	0	0	.	.	.	
.	.	<i>New</i>	.	.	.	1	0	0	
32.572	-116.755	<i>Route modified</i>	1	0	0	1	0	0	
.	.	<i>New</i>	.	.	.	3	0	0	
32.674	-116.863		1	0	0	2	0	0	
32.725	-116.956	<i>Route modified</i>	3	0	0	2	0	0	
32.944	-117.096		3	0	0	3	0	0	
32.737	-116.926		5	0	0	3	0	0	
32.738	-116.663	<i>Retired</i>	1	0	0	.	.	.	
<b>Totals:</b>			133	183	69	139	252	134	
(not including extra visits to Sycuan Peak in 2011)			Visits	Total HC	Max HC	Visits	Total HC	Max HC	



We made a total of 139 site visits during the six week flight season (Table 4), most of which occurred in the four weeks between June 7<sup>th</sup> and July 4<sup>th</sup>. We counted a total of 252 Hermes copper adults (350 if the extra visits to Sycuan Peak are included) distributed across 14 occupied sites. Of the 14 sites with Hermes, only five sites had single day maximum counts of six or more individuals. These were also the only sites that had season-total counts (Pollard Total) of twenty or more (Table 4 and Figure 2).



**Figure 2:** Pollard counts for all sites with Hermes copper butterflies in 2010 or 2011. Pollard counts are the sum of all individuals recorded during the flight season. For Sycuan Peak only a subset of visits were totaled to account for multiple visits made for a mark-recapture study.

Overall there were more Hermes copper adults in 2011 than in 2010, however four sites are responsible for the majority of this observed increase: Sycuan Peak, Loveland Reservoir, Roberts Ranch (North), and Lawson Peak. Lawson Peak experienced a large increase from four total (Pollard) observations in 2010 to 23 observations in 2011. Roberts Ranch also had a similar jump, increasing from five to 20. The Pollard count at Loveland Reservoir increased from eight to 28.

Changes at Sycuan Peak are harder to interpret due to the increased frequency of visits in 2011. Using the midweek (W/Th) visit form 2011, the Pollard count increased from 45 to 58. Interestingly, the maximum *single day count* at Sycuan peak increased from 12 in 2010 to 27 in 2011.

Changes in relative population size are even harder to assess at McGinty Mountain. McGinty Mountain has several routes that are difficult to survey because of access, length, and level of difficulty. We subdivided routes in an attempt to decrease the strenuous nature of the surveys. Unfortunately, we still had trouble surveying these routes during the peak of the flight season due to high temperatures which led to crew fatigue and in one case to heat stress. Despite these differences, we observed a similar maximum count (26 in 2010 and 27 in 2011) and an elevated number of total observations (going from 62 in 2010 to 99 in 2011). In addition we observed a small number of Hermes copper on a face of the mountain where they had not previously been observed.

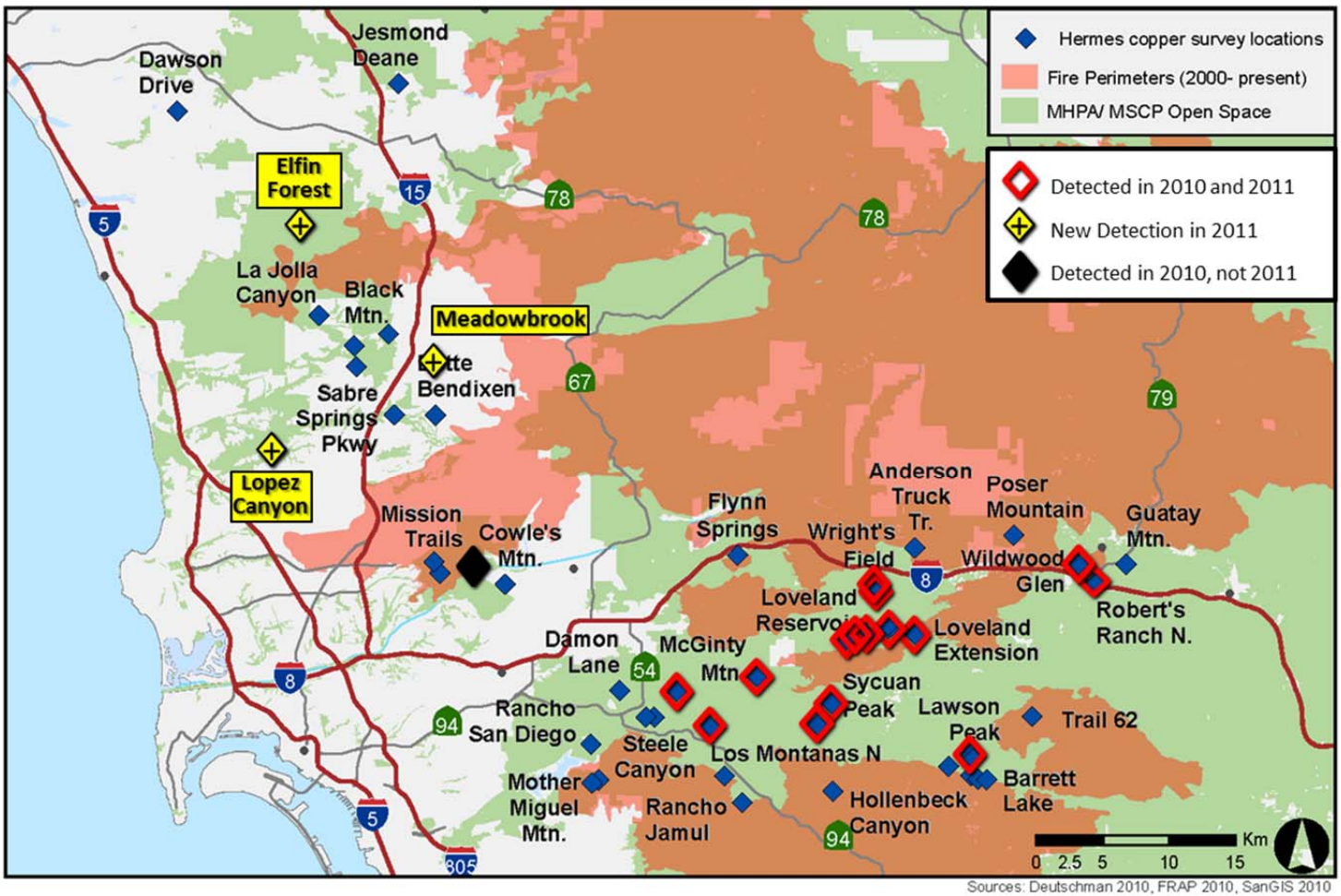
Across all five of these “large” populations, we observed greater numbers of individuals in 2011 than in 2010. However, maximum daily counts never exceeded 30 individuals, even at sites with several long survey routes. This suggests that populations are small. This observation is consistent with the historical literature which comments on the small size of local populations.

Other notable changes in the population status include sites with very small populations. For example, last year we spotted one Hermes copper at Mission Trails Regional Park, but we were unable to find any individuals this year (Figure 3). Last year, we spotted no Hermes copper at the Elfin Forest, however this year we saw one Hermes copper on two separate occasions. The same was true at Meadowbrook Ecological Reserve, where one Hermes copper was sighted this year, but none were apparent in 2010. In addition Michael Couffer with Grey Owl Biological Consulting confirmed the presence of Hermes in Lopez Canyon, which had not been reported since 2008 (Marschalek and Klein 2010). This area is fairly close to the coast and is just west of our sites at Sabre Springs Parkway and Bette Bendixen Park. We observed five individuals at Lopez Canyon in 2011.

Although we looked as far north and west as Vista, our northern most Hermes copper observations were made at the Elfin Forest (Map 3). The new Lopez Canyon site marked the western most detection. We looked as far east as Guatay Mountain, and made our eastern most observation nearby at Roberts Ranch in the Descanso area. We looked as far south as Rancho Jamul Ecological Reserve, our southernmost observation was made at Lawson Peak.

While we did not formally address the effects of temperature and time of day on adult Hermes copper activity, we did make observations that indicate thresholds for both factors. At Sycuan Peak Ecological Reserve, surveys were conducted during a relatively cool day to explore a temperature threshold for adult activity. On June 6<sup>th</sup> a marked male was not observed in its territory at 10:55am and 68.8°F, but was later seen in its territory at 12:35pm and 74.4°F. However, it should be noted that there was a cool wind and instead of exhibiting the normal behavior of patrolling its territory after being spooked, this male landed quickly and began to bask in the sun. On the same day, two other adults were observed with temperatures 65-69°F. After being spooked from their perches they exhibited a limited ability to fly (slower speeds and quickly landing- no patrolling). At this time clouds were periodically blocking the sun, causing temperatures to fluctuate between 67-74°F. This limited activity at 65-69°F was likely due to the sun and warmer temperatures minutes earlier.

On June 23<sup>rd</sup> at Lawson Peak, a warm day allowed for an investigation into the time of initial morning activity. This survey started at 8:25am with a temperature of 78°F, well above the activity temperature threshold. The first Hermes copper was observed at 8:43am (83.8°F). In addition, two Hermes copper butterflies were present at the beginning of the survey route at 10:00am, meaning they were absent around 8:30am. Besides noticing that spooked butterflies tend to leave openings (roads/trails) later in the afternoon, not offering an opportunity to confirm species identification, we have not investigated the time activity stops in the afternoon. Incorporating these observations at Lawson Peak with those on cool days at Sycuan Peak strongly suggests an increased rate of false absences of adult Hermes copper butterflies on surveys when temperatures are below 70°F or the time is before 8:45-9:00am.



**Map 3:** Detections of Hermes copper butterflies on conserved lands, 2010 and 2011. Blue diamonds mark sites with no detections. Red outline indicates that the site was occupied in both 2010 and 2011. New sites are marked in yellow. The only site with a detection in 2010 but not 2011 was Mission Trails.

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## TASK 2 – LANDSCAPE GENETICS

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### USING AFLP MARKERS TO ESTIMATE THE MAGNITUDE OF GENETIC DIFFERENTIATION

#### GOALS AND OBJECTIVES

For Hermes copper, mark and recapture methods are often inadequate for detecting long distance movements. Widely varying temporal and spatial scales, typically low recapture rates, and an inability to determine if an individual has been recruited into the breeding population (even in cases of successful recaptures) create substantial obstacles for such methods. Estimates of genetic variability, combined with inferences of the genetic population structure, provide a means to evaluate the magnitude of differentiation within and among these populations, all of which indicate dispersal ability (gene flow). Increased genetic differentiation suggests that populations are isolated from each other, perhaps even leading to local adaptation. Integrating the genetic data with natural history and landscape features will suggest factors important for the persistence of the species and development of conservation practices. If populations are found to be completely isolated genetically, this would pose radically different policy considerations to conservation efforts than if the populations were all similar.

We used 155 AFLP markers to evaluate the magnitude of differentiation within and among these sampling locations, which indicates dispersal ability (gene flow). We were able to conduct a more comprehensive analysis in 2011 due to larger sample sizes, additional sampling locations and a more complete spatial coverage. Integrating the genetic data with the natural history and landscape features suggests several factors important for the short and long-term conservation of the species.

#### METHODS

We obtained a total of 35 specimens from 12 locations in 2010 (Table 5), but because adult numbers were low at most sites (Deutschman et al. 2010) we were only able to collect single individuals from five of the 12 sampled locations. Collecting specimens from locations which were previously sampled provided the opportunity for temporal comparisons. Samples collected from 2003-2009 [previously analyzed by Deutschman et al. (2010)] were included in some of the analyses.

**Table 5:** Details of Hermes copper specimens obtained in 2010 for genetic analysis in 2011. The table includes location and sample size.

Sampling Location	Sample Size
Lawson Valley	6
McGinty Mountain North	5
Sycuan Peak	5
Bell Bluff Truck Trail	4
McGinty Mountain Northeast	4
McGinty Mountain South	3
Roberts Ranch North	3
<i>California Riding and Hiking Trail</i>	1
<i>Descanso</i>	1
<i>Los Montanas</i>	1
<i>Loveland Reservoir</i>	1
<i>Round Potrero</i>	1
<b>Total</b>	<b>35</b>

## MOLECULAR PROCEDURES

Amplified fragment length polymorphism (AFLP) has the ability to detect genetic variation at the level of individuals for this population-based study (Vos *et al.* 1995). We applied well-understood population genetic models to evaluate the genetic structure of Hermes copper (differentiation among individuals within and between populations) and evidence of dispersal ability. We used the trace analysis program DAX 8.0 to visualize the allelic data; AFLP-SURV (Vekemans 2002, Vekemans *et al.* 2002) to calculate  $F_{ST}$  values; and Geneland (Guillot *et al.* 2005) to investigate spatial genetic structure. Comparing specimens collected in 2010 to those included in Deutschman *et al.* (2010) resulted in a slight adjustment to our method of determining the presence or absence of AFLP markers. The larger dataset presented difficulties when subjectively determining marker presence so we utilized DAX 8.0 to identify markers using a threshold value for the trace signal versus noise level of 4.0.

We calculated  $F_{ST}$  values between the 2010 sampling locations, providing a coarse measure of genetic differentiation as performed in last year's report (Deutschman *et al.* 2010). Comparison of genetic variation within and between populations can provide indirect evidence of movement between populations (sampling locations). A value of zero indicates that individuals from the sampling locations interbreed (completely panmictic population), while a value of one represents completely isolated populations with no gene flow. In rare cases when there are strong tendencies for long-distance dispersal,  $F_{ST}$  values will truly be negative; however, calculations may result in slightly negative values when the true  $F_{ST}$  equals zero. Since most Hermes copper individuals do not exhibit long-distance dispersal behaviors, negative  $F_{ST}$  values were considered equal to zero (no genetic differentiation). One problem with  $F_{ST}$  calculations is that a defined population structure must be determined a priori. Generally, with no prior population knowledge, groups are defined by sampling location. This can be circular as the magnitude of  $F_{ST}$  will be dependent on how one groups the samples to perform the calculation.

To provide a more unbiased view, we used GENELAND 3.3.0 (Guillot *et al.* 2005) to determine clusters of related genotypes because it provides the benefit of integrating spatial coordinates of the samples to define spatial genetic units. Another advantage of using GENELAND over  $F_{ST}$  calculations is that genetic clusters of individuals can be determined (using genotypes) without predefining groupings. Thus, potential biases in specific groupings necessary for  $F_{ST}$  calculations are avoided. Moreover, sites where only one individual was sampled can be used in GENELAND. Once genetic clusters are determined,  $F_{ST}$  can be calculated with more confidence. Other non-spatial clustering models (e.g. STRUCTURE) do not consider the coordinates of the samples. Another benefit is that we are able to include all samples collected from 2003 to 2010 (including samples that are the only specimen from a particular location). As recommended by Guillot *et al.* (2005), we first inferred K (number of genetic clusters) prior to estimating other parameters. The GENELAND software was run 20 times allowing K to vary from 1 to 20 with 1 million Markov chain Monte Carlo (MCMC) iterations, burn-in of 200 iterations, correlated allele frequencies and a spatial coordinate uncertainty of 10 meters. This uncertainty level allows the samples to be assigned to different genetic units and compensates for GPS accuracy. We then ran the same model 20 times with 1 million MCMC iterations at the modal K (K=7) to determine the genetic cluster assignment of individuals.

Each replicate of the MCMC algorithm provides a cluster assignment for each of the 124 individuals in this dataset. In other words, based on the inferred K=7, all individuals will be clustered into at most seven clusters defined by spatially explicit genotypic relationships. Because ghost populations can arise (Falush *et al.* 2003, Guillot *et al.* 2005), and individuals may exhibit a tendency for membership in more than one cluster, replicate runs can vary slightly. Consistent clustering patterns provide support for individual membership in each cluster. To measure this support, pairwise comparisons for cluster

membership of each individual for each run were performed on the 20 replicates. The average linkage (UPGMA) among individuals was calculated and a dendrogram constructed.

## RESULTS

When calculating  $F_{ST}$  values between sampling locations (as was the case in last year's report), locations with only a single individual were excluded from the pairwise  $F_{ST}$  comparisons due the requirement of multiple individuals. Overall, sampling locations show a high degree of similarity (Table 6). However, we detected genetic differentiation between samples from Sycuan Peak and both Bell Bluff Truck Trail and Lawson Valley.

**Table 6:** Pairwise comparison of  $F_{ST}$  from locations sampled in 2010. Data below the diagonal represent pairwise  $F_{ST}$  values with their associated 95% confidence intervals. Data above the diagonal represent pairwise distances (km).

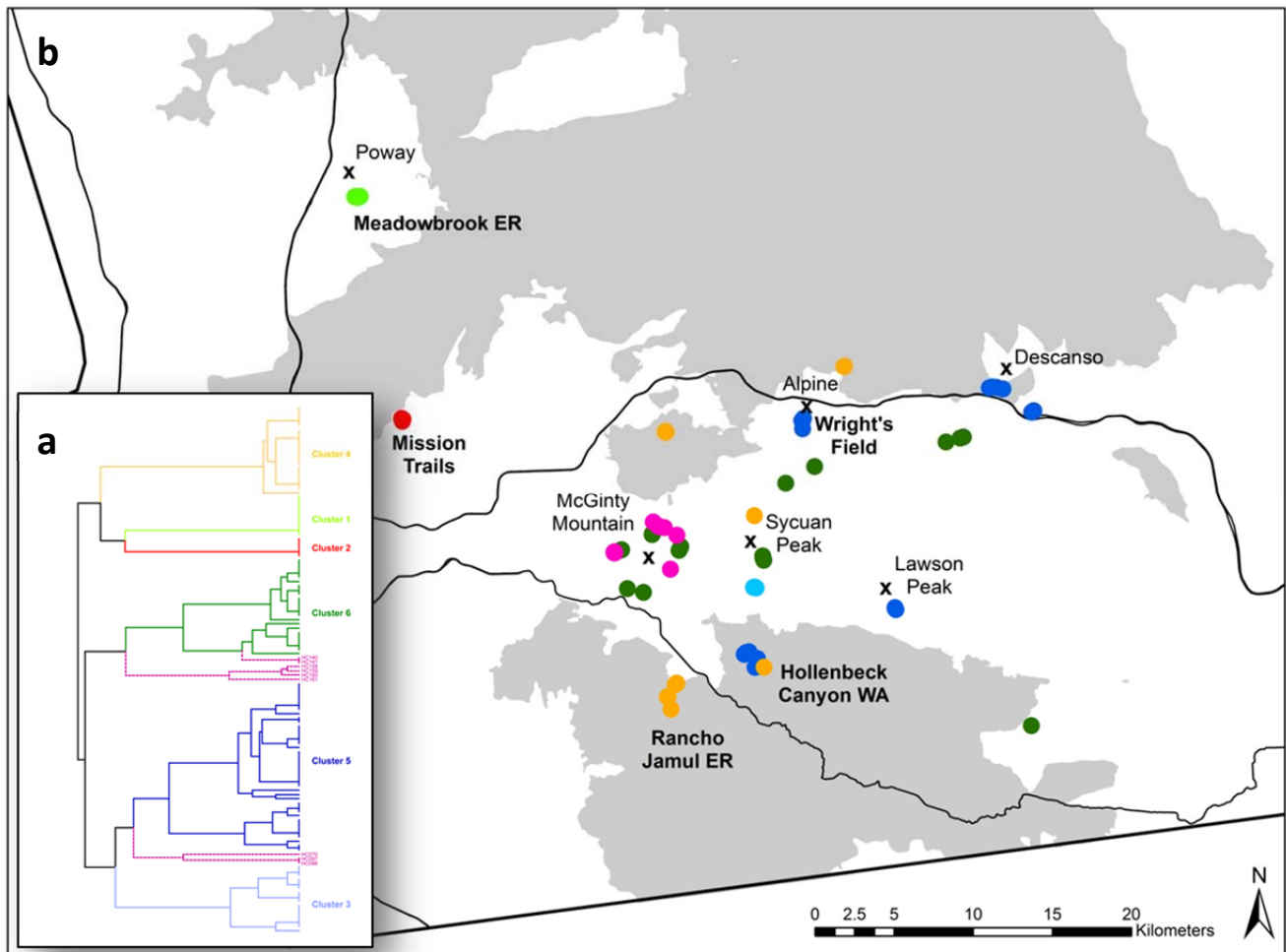
$F_{ST}$	Bell Bluff Truck Trail	Lawson Valley	McGinty Mountain N	McGinty Mountain NE	McGinty Mountain S	Robert's Ranch N	Sycuan Peak
Bell Bluff Truck Trail		16.3	20.6	20.2	19.3	4.7	14.8
Lawson Valley	<b>0.080</b> (-.054, .069)		7.3	7.3	5.3	20.8	1.8
McGinty Mountain N	0.005 (-.058, .050)	0.027 (-.052, .040)		0.6	2.0	25.3	7.2
McGinty Mountain NE	0.112 (-.034, 0.112)	0.036 (-.031, .054)	0.036 (-.040, .039)		2.0	24.9	7.1
McGinty Mountain S	-0.019 (-.063, .053)	-0.005 (-.085, .089)	-0.044 (-.074, .055)	0.091 (-.014, .0918)		23.9	5.3
Robert's Ranch N	-0.031 (-.074, .039)	0.051 (-.042, .080)	-0.012 (-.070, .071)	0.065 (-.028, .065)	-0.001 (-.047, .054)		19.4
Sycuan Peak	0.011 (-.102, .048)	<b>0.066</b> (-.064, .047)	-0.006 (-.069, .020)	<b>0.101</b> (-.030, .080)	0.012 (-.084, .085)	0.081 (-.089, .081)	

Comparing specimens collected in different years at the same location yielded different results. Samples collected at Robert's Ranch North in 2008 and 2010 are genetically similar ( $F_{ST} = 0.099$ , -.076, .099) as well as the samples collected at Lawson Valley in 2008 compared to those of 2009 and 2010 (Table 7). However, statistically significant genetic differentiation was only detected between the 2009 and 2010 samples at this location. These time series comparisons are in areas that did not experience major habitat alteration (e.g. wildfires).

**Table 7:** Pairwise comparison of  $F_{ST}$  from 2008, 2009, and 2010 at Lawson Valley. Data below the diagonal represent pairwise  $F_{ST}$  values with their associated 95% confidence intervals.

$F_{ST}$	Lawson Valley 2008	Lawson Valley 2009
Lawson Valley 2009	-0.019 (-.059, .083)	
Lawson Valley 2010	0.018 (-.040, .047)	<b>0.063</b> (-.070, .046)

Using GENELAND, a spatially explicit analysis including 124 specimens identified seven genetic clusters but only six are consistently supported (Map 4). The lack of a seventh cluster is likely a result of a ghost population appearing in 6 (30%) of our model replicates. Ghost populations are common in the MCMC algorithm for mixture models (Falush et al. 2003). These are areas GENELAND determines as a cluster based on the spatial arrangement of genotypes, but no individuals are assigned to the cluster (Guillot et al. 2005). In fact, most cluster assignments for individuals are consistent among 15-20 (75% - 100%) of the 20 replicates but a few exceptions are discussed below. Due to uncertainty between replicates of the model for some individuals, we were unable to assign some specimens to a particular cluster. These were 9 (47%) of the 19 samples from the slopes of McGinty Mountain.



**Map 4.** Clustering of genotypes by GENELAND. (a) Average linkage (UPGMA) among individuals from 20 GENELAND runs showing the degree of similarity among six genetic clusters. Those individuals unable to be assigned to a particular cluster are indicated with dashed pink lines. Length of lines on the right side is scaled to the number of individuals. (b) Map of Hermes copper genetic clusters assigned by GENELAND. Pink represents individuals that show characteristics of multiple clusters and are therefore unable to be assigned to a particular cluster. The gray shaded region represents wildfire events of 2003 and 2007.

In general, samples from a particular area were very similar or identical in terms of genetic cluster assignment. All individuals from Meadowbrook ER (cluster 1), Mission Trails Regional Park (cluster 2) and Lawson Valley (cluster 3) each represent unique genetic clusters (Map 4). These three locations are the only three sampling locations that represented their own cluster. Specimens from Anderson Road (CNF), Crestridge ER and Rancho Jamul ER are grouped together with one individual sampled at Sycuan Peak ER (North) and Hollenbeck Canyon WA (cluster 4). A fifth cluster (cluster 5) includes the specimens from Descanso, Hollenbeck Canyon WA, Lawson Peak, Robert's Ranch and the two Wright's Field locations. It should be noted that about half of the HCWA individuals show some similarities with cluster 4. The sixth cluster (cluster 6) includes several sampling locations which are found in the McGinty Mountain and Sycuan Peak areas. There is a lack of consistency for genetic cluster assignment for the remaining samples collected on the slopes of McGinty Mountain. For this reason they cannot be reliably assigned to a particular cluster, at least when assessing patterns from 20 replicates.

We discovered evidence of recent dispersal between two genetic clusters. One of 13 individuals from Hollenbeck Canyon WA has the same cluster assignments for all 20 model replicates as all ten Rancho Jamul ER individuals. This is strongly suggestive of a dispersal event, with a distance of about 5.5 km between the two sampling areas. We also found that the two specimens collected at McGinty Mountain ER are intermediately different from each other (10 of 20 replicates support the same cluster) despite the fact that they were collected only 97 meters apart. Ten replicates support the inclusion of one individual in cluster 5 and the other individual with two samples collected at another location on McGinty Mountain.

The clustering results also provide a framework to interpret larger-scale temporal events. First, cluster 4 is composed of specimens from Anderson Road, Crestridge ER, Rancho Jamul ER, one individual from Hollenbeck Canyon WA and one individual from Sycuan Peak ER (North). All of these sites burned in 2003 or 2007 with the exception of Sycuan Peak ER. This suggests that genotype representative of this cluster is found at a lower frequency within Hermes copper than ten years ago. A second observation is that the clusters tend to loosely group individuals collected in the same or preceding year. This is not strictly followed as specimens collected in the same year are assigned to different clusters and specific examples of dispersal events were previously discussed.

Pairwise  $F_{ST}$  comparisons between the genetic clusters show that nine of the 15 (60%) pairwise comparisons were significantly different from zero. This includes all comparisons with cluster 6 which consistently yielded the largest  $F_{ST}$  of all comparisons. Among the statistically significant observations, these results suggest that approximately 7% of the genetic variation observed is due to geographic location. While the magnitude of  $F_{ST}$  seems small, interpreted in context with the limited range of the species, such magnitudes of genetic differentiation are not trivial. Thus, even though the range of Hermes copper is small, the frequency of dispersal over the landscape still remains limited. The reason GENELAND is able to define clusters that are not always differentiated using  $F_{ST}$  calculations is that GENELAND is a more sophisticated model that incorporates spatial data and correlated allele frequencies to determine the distribution of expected genotypes;  $F_{ST}$  values are a coarse measure of genetic differentiation determined only by allelic variation.



**Table 8:** Pairwise comparison of  $F_{ST}$  from clusters defined by Geneland. Data below the diagonal represent pairwise  $F_{ST}$  values with their associated 95% confidence intervals.

$F_{ST}$	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1						
Cluster 2	0.033 (-.069, .045)					
Cluster 3	<b>0.063</b> (-.037, .032)	<b>0.050</b> (-.047, .046)				
Cluster 4	0.024 (-.034, .043)	0.027 (-.048, .066)	<b>0.027</b> (-.026, .026)			
Cluster 5	<b>0.046</b> (-.024, .037)	0.024 (-.037, .062)	0.025 (-.016, .026)	0.021 (-.013, .022)		
Cluster 6	<b>0.122</b> (-.032, .034)	<b>0.147</b> (-.043, .063)	<b>0.066</b> (-.023, .023)	<b>0.086</b> (-.021, .025)	<b>0.077</b> (-.012, .021)	

## DISCUSSION

Areas of restricted movement are indicated by the presences of significantly different  $F_{ST}$  values (Deutschman et al. 2010 and this report) and the identification of genetic clusters. Peripheral sampling locations such as Meadowbrook ER and Mission Trails Regional Park warrant further investigation because all individuals sampled in these areas belong to their own genetic cluster. This suggests that immigration and emigration events are less frequent at least when compared to other localities/clusters which appear to be more widely distributed. While several years of data support the connectivity of occupied habitat patches in the eastern area, we have not been able to further evaluate more peripheral locations since the 2003 samples mainly due to wildfires leading to local extirpations (Marschalek and Klein 2010). Based on our previous investigations, these areas are more likely to be differentiated genetically from other locations and are underrepresented in our sampling. Specifically, Lopez Canyon is a location that should be sampled as this represents a currently occupied habitat patch in a region not yet included in our genetic analyses.

Evaluating the pairwise  $F_{ST}$  comparisons (Table 6) for Hermes copper suggests that little genetic structure was present in the 2010 sampling location. However, the 2010 sampling locations were obtained from an area with relatively less development and continuous coastal sage scrub habitat. This is the same area which our previous analysis also suggested increased connectivity of sampling locations (Deutschman et al. 2010). Spatial patterns of the genetic clusters also support increased dispersal (compared to peripheral sampling locations) as three clusters (4, 5, 6) extend through this area. Many of the individuals collected from the slopes of McGinty Mountain were not consistently assigned to a particular cluster due to their genotypes sharing similarity with several other clusters. This could result from individuals dispersing into this area from multiple clusters, again suggestive of increased movement within these particular portions of the landscape.

As we found in previous analyses (Deutschman et al. 2010), our current analyses suggest the possibility of a temporal component influencing the individual makeup of Hermes copper in San Diego County. This is evident by changes in the magnitude of genetic differentiation between years even given no obvious changes in habitat. Another important result from the temporal sampling is that we discovered

that one genotype represented by cluster 4 was nearly extirpated by the fires of 2003 and 2007. Increasing sample sizes for these temporal investigations is particularly important to assist in identifying migrants and changing genetic compositions of local populations.

Our initial investigation (Deutschman et al. 2010 and this report) into the landscape genetics of Hermes copper suggests caution when interpreting the  $F_{ST}$  values as the estimates were variable depending on the criteria used to determine AFLP marker presences/absence. This is likely due to the smaller sample sizes which may produce higher  $F_{ST}$  values (Medina et al. 2006). In addition, overall genetic variation of a local population may not be fully represented in the samples, and any presence or absence of a marker greatly changes the allele frequency.

Advantageously, analyses in this report include a spatially explicit model which clusters all samples while reducing investigator bias from arbitrarily grouping individuals as  $F_{ST}$  calculations require. This spatial model also allows us to detect dispersal events more easily.

For these reasons, we feel that only these very broad conclusions should be considered at this point:

1. The occupied patches of redberry in the eastern part of the Hermes copper range between Jamul and Descanso are relatively well connected by Hermes copper dispersal. This is based on consistently low (or no) genetic differentiation between these sampling locations over several years.
2. Genetic differentiation and the identification of genetic clusters suggest that movement of individuals is restricted in parts of the landscape, particularly on the periphery of Hermes copper's distribution.
3. While defined clusters could be identified, all sampled individuals are generally similar to each other based on low polymorphism rates, as many of the AFLP markers (39 of 155; 25%) are monomorphic (shared by all individuals).
4. Increased sample sizes are required for reliable genetic differentiation estimates at a fine spatial scale. Increasing the number of AFLP markers by using additional primer sets could also benefit these analyses.

## NON-LETHAL GENETIC SAMPLING

### GOALS AND OBJECTIVES:

Due to the small number of Hermes copper adults observed during the last decade and the concern about the status of the species (Marschalek and Klein 2010, Deutschman et al. 2010, USFWS 2011), we decided to explore nonlethal sampling for genetics research. In general, most research and associated statistical analyses benefit from a greater sample size. However, doing so is contradictory to fundamental conservation ideals when sampling requires the killing of individuals, particularly in extremely rare species such as Hermes copper. Most research regarding non-lethal genetic sampling focuses on obtaining enough DNA from an organism to generate a reliable genetic marker. However, much less understood is whether or not capturing and/or sampling impacts the survival of the individuals.

We wanted to investigate three aspects of conducting population genetics research with AFLPs on Hermes copper. This includes 1) determining how much DNA is required for replicable AFLP data, 2) how much DNA can be obtained from a leg or portion of a wing, and 3) if removing a leg from an individual impacts its survival (survival following wing sampling was not tested).

### DNA YIELD FROM LEGS AND WINGS

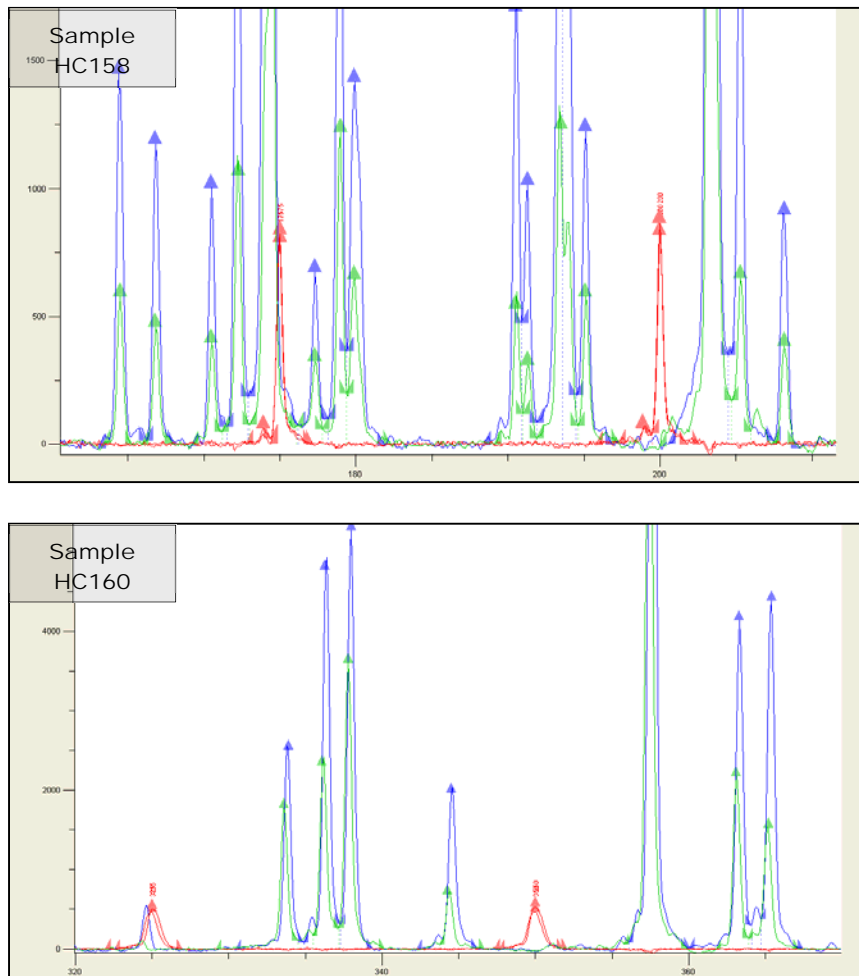
**Methods:** We used legs and wings of specimens from which we had already isolated DNA from the remainder of the body and incorporated in the 2010 analyses (Deutschman et al. 2010). For DNA isolation from a single Hermes copper leg, methods tested include 1) CTAB (Moller (1992) following Reineke *et al.* (1998)), 2) DNeasy Blood and Tissue Kit (Qiagen), 3) Squishing Buffer (10mM Tris, 1mM EDTA, 25mM NaCl, Gloor et al. 1993), and 4) DNeasy Kit with Squishing Buffer. We conducted two replicates for each treatment, with the exception of CTAB which only one sample was tested. The treatments involving the Qiagen kit were followed by a sodium acetate/ethanol precipitation step to concentrate the DNA sample. The AFLP marker data obtained from the whole body DNA isolations were the standard for which AFLP marker data derived from leg and wing isolations were compared to determine accuracy and replicability

**Results:** DNA isolation with CTAB using a single leg was not effective as no DNA was detected by the spectrophotometer or visually on an ethidium bromide 1% agarose gel. Because there was no detectable DNA, no efforts to concentrate the sample were performed. Using the Qiagen DNeasy Kit (with and without Squishing Buffer), we were able obtain DNA with all samples containing well over 200ng of DNA (Table 9). The method utilizing only Squishing Buffer does not have a separate DNA purification step but proceeds directly to the AFLP process. For this reason we were unable to determine an accurate starting amount of DNA present in the sample but still used it in the AFLP procedure.

**Table 9.** Methods to obtain DNA from one Hermes copper leg.

Method	Sample and Total DNA Yield (ng)	Result
CTAB	HC106: undetectable	Did not proceed to AFLP procedure due to insufficient DNA yield.
Squishing Buffer	HC124, HC127: unknown- proceeded directly to digestion	Minor differences when compared to AFLP marker data of whole body DNA extraction.
Qiagen Kit + Squishing Buffer	HC141: 540 HC156: 1060	Minor differences when compared to AFLP marker data of whole body DNA extraction.
Qiagen Kit (follow kit protocol)	HC158: 1600 HC160: 2200	Trace data matches AFLP marker data from whole body extraction.

Both methods involving Squishing Buffer produced AFLP marker data that were very similar to the marker data using whole body DNA extractions, but there were minor differences in the number of markers presence (Not Shown). Relative heights of samples differed due to different amounts of starting DNA and amplification efficiency, but are not important for our purposes; our goal was to simply score marker presence and absence. Using the Qiagen DNeasy Kit (alone) following standard protocol resulted in trace data from a single DNA extraction that matched the whole body extraction trace (Figure 3).



**Figure 3.** Relative fluorescence intensity (RFI) for DNA from a single leg compared to the whole body. DNA was extracted from a leg using Qiagen Kit (blue) and the whole body (green). Although the heights vary, all AFLP markers are present in both samples.

DNA yields from half of a wing (HC106) using CTAB were too low for further testing. We felt that using a smaller portion of a wing that would have minimal impact on flight ability would result in DNA yields much less than those obtained from a leg. Using half of a wing (HC106) with the Qiagen Kit followed by an ethanol precipitation yielded plenty of DNA for the AFLP process using standard protocols. Starting with a restriction digestion with 4ul of concentrated sample (approximately 320 and 200ng DNA) demonstrated the replicability of the AFLP process, both among the DNA isolated from wing and between wing and whole body DNA isolation.

The Qiagen DNeasy Kits using legs and wings provided the highest DNA yields of sufficient quality to obtain replicable AFLP data. We did not investigate DNA yields from smaller wing portions because we felt that obtaining legs from adults would result in less impact compared to wing sampling. Removing half of a wing would certainly impact the mobility (and possibly survival, mating success and territory defense) so comparing DNA yields from smaller portions should be investigated prior to any wing sampling for AFLP studies.

## SERIAL DILUTIONS

**Methods:** We chose one Hermes copper sample (HC75) that would have ample genetic material for a high number of reactions. The sample was serially diluted to run reactions starting with 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.5, and 0.025ng. Reactions followed the same protocol as above which includes 25 preselective PCR cycles. Because each cycle will theoretically double the amplified genetic material, we also increased the number of preselective PCR cycles by one for each halving dilution (Table 10). This is an attempt to standardize the final quantity of amplified DNA fragments. We ran 8 replicates for each starting DNA amount with 25 and adjusted number of preselective PCR cycles. Using 200ng of DNA and 25 preselective PCR cycles was used as the standard to compare against the other treatment levels. We assessed replicability within and between treatments by identifying missing or new markers.

**Table 10.** Serial dilution of starting amounts for AFLP procedure. A list of starting DNA amounts for the AFLP procedure, with the number of preselective PCR reactions to standardize the final amplified DNA output. Two-hundred ng of starting DNA and 25 preselective PCR cycles is standard protocol.

<b>Starting DNA (ng)</b>	200	100	50	25	12.5	6.25	3.125	1.5625	0.5	0.025
<b>Preselective PCR Cycles</b>	25	26	27	28	29	30	31	32	34	38

**Results:** We found that both the amount of starting DNA and the number of preselective PCR reactions affect the presence of AFLP markers (Table 8). With 25 preselective PCR reactions, similar numbers of markers are present in all replicates (consistent markers) using at least 25ng DNA. Smaller amounts of starting DNA reduced the numbers of these markers. Increasing the number of PCR reactions increased the number of consistent markers for starting DNA amounts less than 25ng, including similar numbers from 200 to 0.5ng DNA. Using up to 40 PCR reactions with 0.025ng DNA still resulted in substantially lower numbers of consistently amplified markers.

**Table 11.** Assessment of the starting DNA amount and number of preselective PCR reactions relating to the presences of AFLP markers. The number of AFLP markers represents the number of markers that were present in all replicates for each treatment. We did not assess treatments starting with 0.5 or 0.025 ng DNA for 25 preselective cycles.

<b>Starting DNA (ng)</b>	<b>Preselective PCR Reactions</b>	<b>AFLP Markers</b>	<b>Preselective PCR Reactions</b>	<b>AFLP Markers</b>
<b>200</b>	25	112	25	112
<b>100</b>	25	112	26	110
<b>50</b>	25	110	27	106
<b>25</b>	25	105	28	108
<b>12.5</b>	25	77	29	93
<b>6.25</b>	25	28	30	98
<b>3.125</b>	25	1	31	101
<b>1.5625</b>	25	0	32	103
<b>0.5</b>	25	--	34	98
<b>0.025</b>	25	--	38	7
<b>0.025</b>	25	--	40	23

We found that one leg from an adult Hermes copper butterfly provides enough DNA for replicable AFLP results. In fact, the results suggest that even a portion of a leg could provide enough DNA, and the number of preselective cycles could be increased in cases of suboptimal DNA yields. Because these results indicate that a single leg can be used for an AFLP sample, we proceeded to determine if removing a leg would impact the survival of adults.

Calculating the exact minimum DNA amount required for the AFLP procedure is not clear cut, as reducing the starting DNA amount tends to decrease the number of AFLP markers present. It is unclear what impact missing peaks will have on genetic analyses, especially since we observed some peaks dropping out of all replicated for a certain treatment level. In addition, certain activities can be implemented to obtain reliable trace data with reduced DNA quantities. Conducting replicates with lower starting DNA will help assure that at least one replicate amplifies well and would also serve to confirm presence/absence of all peaks. It should be noted that we limited our analyses to increasing the preselective PCR cycles based on each cycle being 100% efficient in amplification. This is generally not the case in practice and increasing the cycle numbers could in fact further increase the consistency of amplifying all AFLP markers.

## MARK-RELEASE-RECAPTURE

**Methods:** We conducted a mark-release-recapture study at Sycuan Peak Ecological Reserve to assess the impact of presumed nonlethal sampling on adult survival and longevity. We uniquely marked all captured adults with a felt-tipped marker (Ehrlich and Davidson 1960) and recorded locations of all Hermes copper sightings with a handheld GPS unit. In addition, we removed one leg from half of the males (as a genetic sample) captured during each survey. We found that the front (prothoracic) legs were more easily pulled off compared to the other legs. Survival is assessed by resighting rates. Longevity represents the minimum number of days an individual was known to be alive; however, this likely underestimates the actual longevity because individuals may leave the survey area and surveys were not conducted daily. Only those males that were resighted are included in the longevity analyses because those not resighted likely left the area rather than died. This assumption is based on recapture rates from more complete marking studies (Marschalek and Deutschman 2008). Females were excluded from leg removal due to low resighting rates of females (Marschalek and Deutschman 2008, Marschalek and Klein 2010) and our desire to limit the impact to females by not removing a leg.

**Results:** A total of 72 adult Hermes copper butterflies were marked, 56-58 males and 14-15 females. Due to the lack of sexual dimorphism other than females with a swollen abdomen (Figure 4), there is some uncertainty in determining the gender for a couple individuals. A comparison of males only marked (6-Legs) and those marked with a leg removed (5-Legs) shows a slightly higher resighting rate for the 5-Legs group and a slightly higher lifespan for the 6-Legs group (Table 12). None of these differences were statistically significant. As in previous years at other sites, females were rarely resighted.

We did not assess the impacts of marking and/or leg removal on behavior of Hermes copper adults. However, both 5-Legs and 6-Legs males were observed maintaining the same territory for a span of several days. In addition, less than two hours after marking and removing a leg from a specific male, he was observed mating with a female (Figure 5).



**Figure 4.** Photo of a male (left) and female (right) *Hermes copper* while captured in a net. Males are identified by a relatively long and narrow abdomen while females have a wide abdomen.

**Table 12.** Resighting rates and longevity of *Hermes copper* from a mark-release-recapture rate at Sycuan Peak Ecological Reserve. We compared those only marked with those marked and one leg removed. Only resighted individuals are included in the longevity data.

	Number Marked	Number Resighted	Avg Min Lifespan	Avg Max Lifespan
All Females	14	1 (7%)	5	10
All Males	58	30 (52%)	6.6 (±3.5)	10.0 (±3.4)
Males 6 legs	31	14 (45%)	7.0 (±3.8)	10.4 (±3.8)
Males 5 legs	27	15 (56%)	6.3 (±3.5)	9.8 (±3.2)
		$\chi^2 = 0.62$	t = 0.73	t = 0.65
		p = 0.430	p = 0.468	p = 0.517



**Figure 5.** Photo of a male (bottom) and female (top) *Hermes copper* mating at Lawson Peak. The male was marked and had one leg removed less than two hours prior to being observed mating.

Discussion: In the past, few studies have investigated the impact of non-lethal genetic sampling for insects (Starks and Peters 2002). However, in the last two years several published papers demonstrate that either wing or leg sampling from butterflies provides sufficient DNA and does not alter the behavior or survival of the individual (Keyghobadi et al. 2009, Hamm et al. 2010, Koscinski et al. 2011). The butterfly species chosen for these studies are larger than *Hermes copper* and would presumably be easier to handle and to remove tissue from an individual without negative consequences.

Our study shows that a well-trained person can capture and remove a leg from an adult *Hermes copper* without affecting its survival. The leg will also provide DNA of sufficient quantity and quality for AFLP research. This is particularly important for investigating the landscape genetics of *Hermes copper* as this species is found in limited locations and generally few individuals are observed at these locations.

It is accepted that the AFLP procedure requires a greater amount of starting DNA than other genetic techniques such as microsatellites and mtDNA. However, few studies have looked at the minimum amount of DNA required for AFLP (Vos et al. 1995, Coyle et al. 2005, Indsto et al. 2005). We found that 100ng of starting DNA provides the same results as 200ng using our standard method. However, this study demonstrates that smaller amounts of DNA can be used effectively with specific modifications to the standard protocol. In population-level studies we suspect that minor marker dropout would have minimal effects and likely will not impact the *relative* values of any genetic calculation. Although more analytical research must be first performed, we already recognize that such impacts would be more pronounced with smaller sample sizes. This is highly relevant to non-lethal sampling efforts as acquiring even less material should correspondingly result in even further reducing impact. This particularly important finding not only applies to *Hermes copper* research, but also to other rare species which are smaller in size and not as strong fliers.

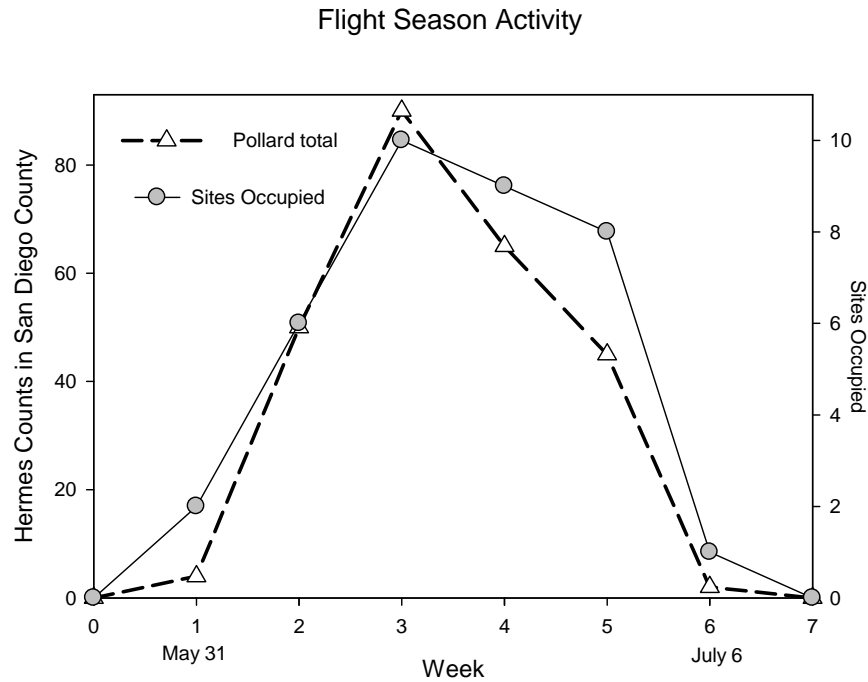


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## TASK 3 – SYNTHESIS AND COMPREHENSIVE ANALYSIS

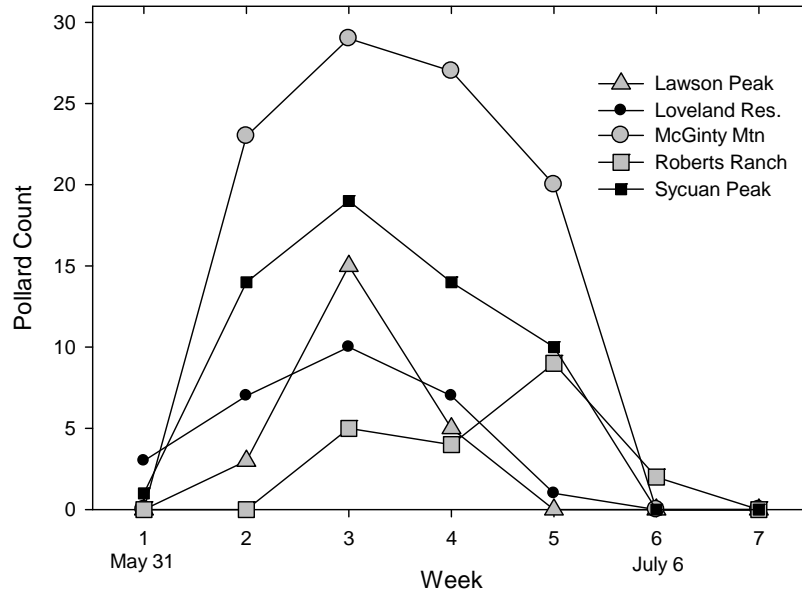
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We began sampling when the first Hermes copper adult emerged at Sycuan Peak. Only Loveland Reservoir also had butterflies that week, however counts increased rapidly over the next two weeks. By the week of June 14<sup>th</sup> (through the 20<sup>th</sup>), we detected Hermes copper at 10 sites (Figure 6, gray circles). We also observed peak densities totaling more than 80 individuals the same week (Figure 6, white triangles). Adult counts decreased gradually from there.



**Figure 6:** Hermes copper distribution and population size through the flight season. Data are summarized weekly.

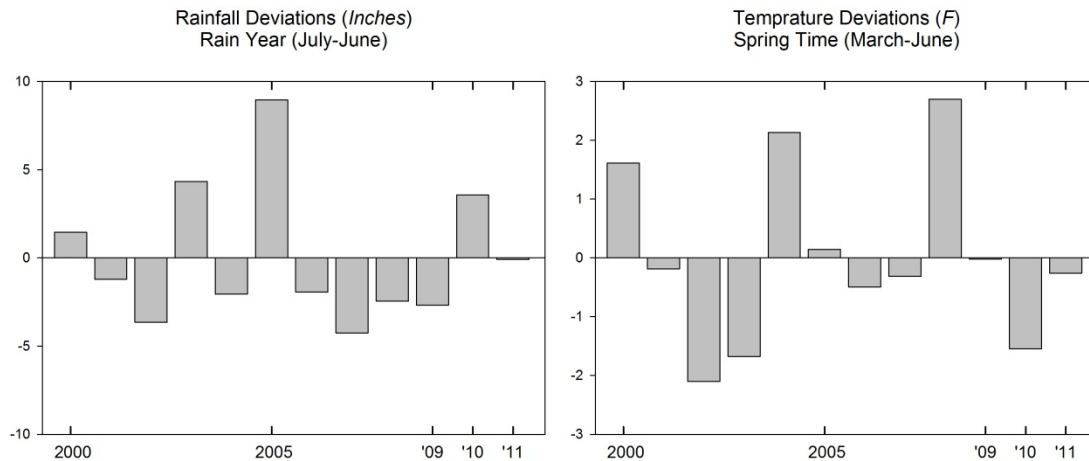
The pattern of a sharp increase in adult numbers during the beginning of the season and a gradual decline after the season peak is fairly typical for butterfly populations; however, this pattern can be variable (Figure 7). The sites with the smallest daily counts tended to have the shortest flight season, indicating that only a few individuals ever emerged at those sites (as opposed to some emerging for many consecutive days).



**Figure 7:** Hermes copper distribution and population size at Lawson Peak, Loveland Reservoir, McGinty Mountain, Roberts Ranch, and Sycuan Peak. Data are summarized weekly.

Marschalek and Deutschman (2008) show that Hermes in captivity have a threshold for becoming active around 22°C (72°F) and field observations indicate adults tend to seek shade in the vegetation at high temperatures (above 90°F). This year we recorded specific environmental information for 347 Hermes copper observations, and of those only 5 were made below 72 degrees (~ 1.5%). All Hermes copper were active between 68.7 and 95 degrees. The median active temperature we observed was 82 degrees

In addition to being cool, 2010 was the first above average rainfall year since 2005 (as measured at the Otay Lakes weather station which is close to the center of the historical Hermes range) (Figure 8). The years of 2007, 2008 and 2009 were between two and four inches below average. Although periods of drought are frequent in San Diego County, the window between 2006 and 2009 represents the longest dry period over the last 12 years (since 1999). Following the above average rainfall year of 2010 we did see higher densities in Hermes copper populations. This observation is consistent with other research that has clearly demonstrated the importance of precipitation to adult butterfly numbers (Pollard 1988, Roy et al. 2001).



**Figure 8:** Deviations from average rainfall (left) and maximum temperature (right) at Otay Lakes, San Diego, CA. Rainfall anomalies are based on July of the previous calendar year through June totals. Temperature anomalies are based on March through June values.

Although we do not have enough data to explain why Hermes populations were larger in 2011 than 2010, we can draw some conclusions about the distribution of Hermes copper across the sites we visited. We estimated the concordance of site occupancy between 2010 and 2011. Concordance is based on the number of sites that were the remained in the same state in both years (both sites occupied in 2010 and 2011 and sites that were unoccupied in both years) compared to the number of sites that changed status (occupied in 2010 but not 2011 or unoccupied in 2010 but occupied in 2011). The index is based on the proportion of concordant sites minus the proportion of discordant sites and can range from +1 (perfect concordance) to -1 (perfect discordance). Values near 0 indicate no relationship between occupancy in 2010 and 2011.

Our measure of concordance was high (0.81, Table 13). Only three sites had a change in their occupancy. In 2010, a single Hermes copper was sighted on Kwaay Paay Peak in Mission Trails Regional Park (an area not burned recently), but we were unable to find adult Hermes in that area this year. In 2011 we spotted a single butterfly at Meadowbrook Ecological Reserve once, and another single individual at Elfin Forest twice, both in areas where we found none in 2010. In all three cases the number of butterflies in question is small; single individuals spotted once or twice.

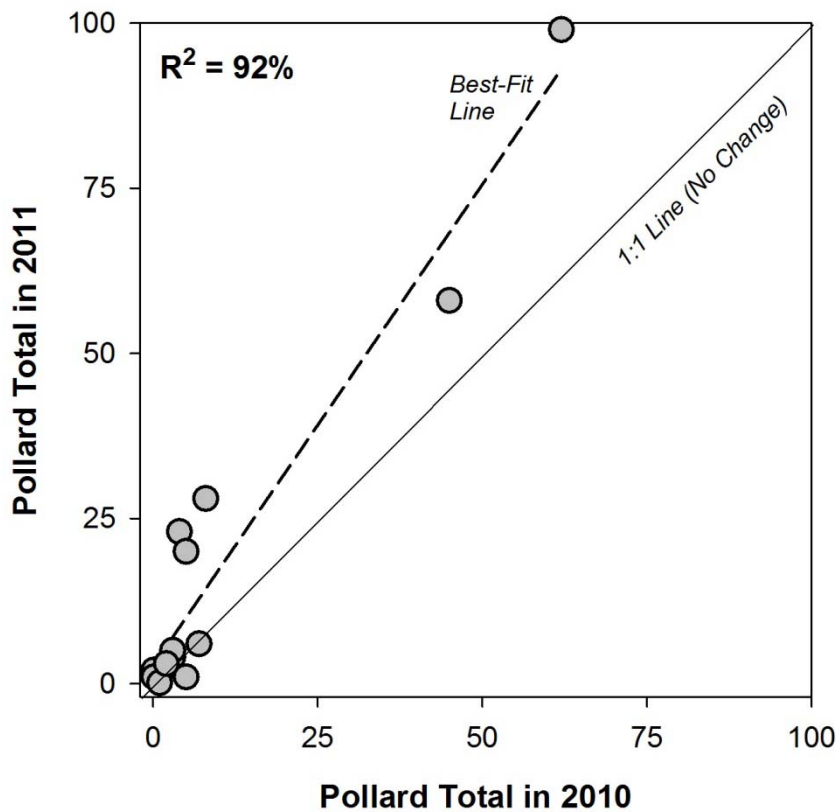
		2011	
		Occupied	Unoccupied
2010	Occupied	11	1
	Unoccupied	2	17
<b>Measure of Concordance</b>		<b>0.81</b>	

**Table 13:** Concordance of populations surveyed in 2010 and 2011. Concordance is a measure of similarity that can range from -1 (completely discordant, status changes at all sites) to +1 (completely concordant, status does not change at any site). A concordance of 0 represents no relationship (number of sites that change is equal to the number that do not change)

Most sites which were occupied in 2010 remained occupied in 2011. The larger populations in 2010 were also the larger populations in 2011. Although we only have two years of data, the high level of concordance between 2010 and 2011, as well as anecdotal evidence, suggests that Hermes copper populations reoccur at the same places year after year (except following fire).

We also estimated the change in relative population size. Although the pattern of occupancy did not change in 2011, the relative population sizes were larger at most sites in 2011 (Figure 9). In this figure, sites lying along the solid line showed no change in population size and sites above the 1:1 line indicate an increased population size. In general, modest sized populations didn't change, or saw a slight increase in population size (usually by 1 or 2 individuals). Larger populations tended to see much more dramatic increases in terms of absolute numbers.

The relationship between population size among occupied sites was positive and very strong ( $R^2 = 92\%$ ). This means that in addition to concordance being high (populations stay where they are), that large populations tend to stay large and small populations tend to stay small.



**Figure 9:** Hermes population sizes in 2010 and 2011, analyzed by site. Only sites which were surveyed both years and occupied are shown (n=14). Data are shown on a linear scale.  $R^2$  is given from the standard regression line (dashed line). The solid line (1:1 line) indicates no change from 2010 to 2011.

## HERMES POPULATIONS

We documented Hermes copper at 14 of 34 sites that were identified as potential high-quality habitat. Most of these occupied sites had less than 10 total butterflies observed over the entire flight season and single-day maximum counts under five. In total, we counted 350 individuals over the course of 117 site visits county-wide (these data includes heavy sampling of Sycuan Peak). Although this represents an increase from 2010, it is still a relatively small number of individuals. More alarmingly, the five largest populations all lay within the same strip of unburned habitat stretching from Descanso to the Jamul/Rancho San Diego area. If that small area were to burn simultaneously in a Santa Ana wind-blown fire event, the total number of sites with recent Hermes sightings would be reduced to three.

This year's data has allowed us to confirm anecdotal reports that Hermes copper populations do not seem to move from year to year. We have two years of intensive survey data confirming that a large number of sites with seemingly suitable habitat are not occupied by Hermes copper. We also know that small populations tend to stay small and large populations tend to stay large, but that the absolute population size can fluctuate somewhat year to year.

These results have implications for future Hermes copper monitoring procedures. The probability Hermes copper appears at sites which were unoccupied for consecutive years is low. As a result it is important to evaluate the cost/benefit of revisiting these sites. The same is true with large, robust populations. We expect to observe adults in the same areas from year-to-year, so effort spent counting individuals may be better spent answering some of the remaining questions about their behavior, biology and habitat preferences. It seems like an appropriate time to resolve critical uncertainties about the species, including dispersal ability, female behavior and reproductive processes, larval habits and developing a protocol to rear the butterflies in a laboratory setting. It is crucial to direct our attention to broadening our understanding of the species, prioritizing conservation actions and establishing last-resort means for preserving the species.

The results from our 2010 and 2011 field surveys suggest that Hermes copper populations are primarily limited to a small portion of San Diego County. This area is substantially smaller than the historic range of the population. There is ample cause for concern for the future of the species. As a candidate species, Hermes copper will eventually receive protection under the ESA with the creation of a formal recovery plan. In preparation for that process it is important to resolve as many critical uncertainties about the species as possible. This includes understanding suitable Hermes copper habitat, the species' reproductive process, dispersal ability, and the minimum number of Hermes needed in a given area to create a stable population. In addition, an insurance policy against fire, in the form of in vitro rearing could be critical to the persistence of the species in the United States.

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## DISCUSSION: CONCEPTUAL MODEL AND CRITICAL UNCERTAINTIES

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One of the most important steps in conservation monitoring and management is to construct a conceptual model that reflects best available scientific knowledge about the species as well as monitoring and management objectives. We have created an initial (draft) conceptual model following the guidelines described in Hierl et al. 2007 (Figure 10). Hierl et al. emphasize the importance of parsimony in models and stress that they should reflect relationships that are well documented. In our model, we included dashed lines for relationships inside Hermes copper life history (green circle) that we know must exist, but which we know little about. As the main goal of this model is to set the stage for research which supports the development of a recovery plan, these uncertainties are of prime interest. In addition we have added grey boxes not connected to the model directly to indicate the large number of uncertainties that exist in the system, but which are lower in priority than clarifying the life history of the species.

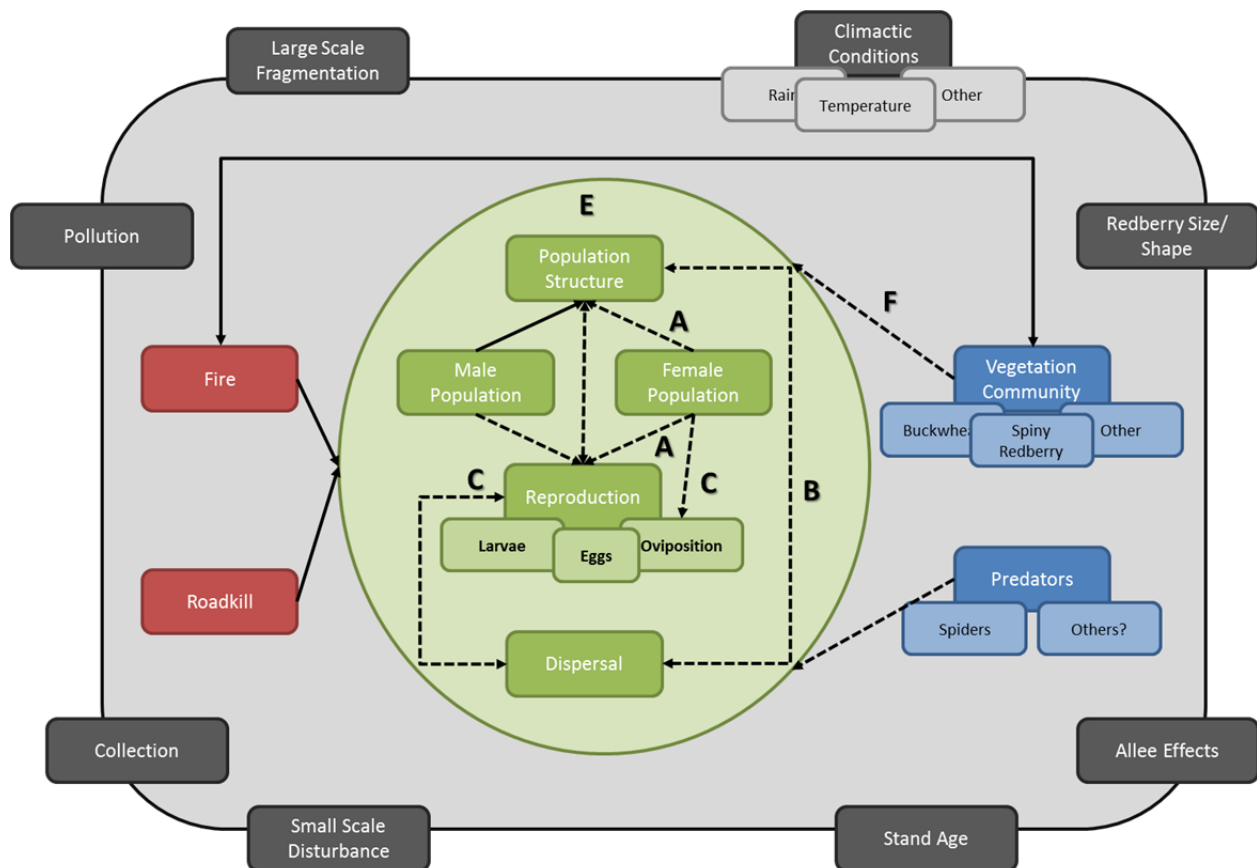
The basic life history of Hermes copper is fairly well understood (Marschalek and Deutschman 2008, Marschalek and Klein 2010). The vegetation community plays a role by supporting the larval and egg host plants, and nectar plants. Fire is a large and potentially catastrophic force acting on Hermes copper populations. Assessing vegetation community requirements and dispersal potential could allow us to estimate the time needed for the species to encroach in areas devastated by fire. We have direct evidence showing that spiders can kill Hermes copper (and suspect other taxa may do the same), but it is unclear how important predators or parasitoids may be to the persistence of Hermes copper. Roadkill has also been indirectly observed, but like predation it is unclear how important this stressor may be.

### A. FEMALE BEHAVIOR

Hermes copper adult males are territorial and are therefore easy to capture, track, and re-sight. Female Hermes copper, on the other hand are relatively elusive as we observe fewer females than males and they tend not return to the same area if spooked. This difference in behavior make estimating the true population size of Hermes copper difficult. Mark-recapture, or curve fitting methods like INCA are unreliable. Marschalek was able to show that total Pollard and max counts were stable methods for estimating population size, but was not able to study female dispersal ability, range and habits. Filling in these information gaps is important because it will help us refine our population estimates as well as estimate the potential for natural dispersal between sites. There have been few observations of females in the field. As reproduction is the crux of a species' persistence, it is critical to understand all that we can about this process. At this time, details of Hermes copper reproduction is poorly known. This information could help us define what constitutes high quality, critical habitat.

### B. LANDSCAPE GENETICS

At this time the best option we have for quantifying the dispersal ability of Hermes copper is through genetic analysis. Genetic analysis also holds the key to determine if populations are mixing frequently enough to avoid inbreeding and genetic bottlenecks which can reduce fitness, and will allow us to define what constitutes a distinct population. This year we confirmed that enough DNA could be extracted from a single leg to sequence an individual. We also demonstrated that this method of taking a single leg was non-lethal and did not reduce an individual's movements or lifespan.



**Figure 10:** Draft conceptual model for *Hermes copper*. Elements in green and inside the green arrow represent aspects of the *Hermes copper* species which should be monitored or investigated in order to understand what conservation efforts should be and later, if they are working. Blue boxes represent natural drivers which can only be managed in limited ways by people. Red boxes are anthropogenic stressors which in some cases are manageable directly. Grey boxes on the edge of the figure represent other uncertainties which cannot be explored until more life history traits are resolved. Dashed lines indicate critical uncertainties about *Hermes copper* life history which must be resolved before other progress can be made in conservation management and research. Solid lines represent well established facts and/or relationships.

### C. REPRODUCTIVE PROCESS AND LARVAL HABIT

Another key to understanding population size and fluctuations is the behavior of larvae. For instance, it is unclear if larvae are capable of secondary diapause during unfavorable years. Larvae were extremely difficult to find in the field when Thorne was describing the species in 1963, when there was a far larger population. We do not know what conditions must be met for eggs to hatch, cause larvae to pupate or go into diapause (if indeed they do). We also don't know if habitat defined by adult male territories is ideal for larvae. The larva stage is another part of the reproductive process that could hold information critical to conservation.

### D. LABORATORY REARING

Although it is undesirable to achieve conservation by keeping populations out of the wild, rearing *Hermes copper* in vitro may be an important insurance measure against catastrophic fire in the single densely populated portion of the county. In 1963 Thorne noted that *Hermes copper* was difficult to rear out of the wild. This may continue to be true, however, perhaps with the information gleaned by studying

females, eggs and larvae we can establish more positive outcomes and develop a procedure for rearing Hermes copper successfully.

#### E. SMALL POPULATIONS

The majority of Hermes copper populations are small. This year we noted three sites that changed their occupancy status by adding or removing a single individual. It is unclear if these populations are especially small but stable or if they are in the transient detections. In addition, we do not know, for example, if Hermes copper suffer reduced reproduction because individuals are either located too far from one another or if local butterflies are inbred. Interestingly, all the sites which changed occupancy status are further north than, and disconnected from, the core area where most Hermes copper are found.

#### F. HABITAT REQUIREMENTS

We currently have limited information about why some areas with abundant spiny redberry and buckwheat are unoccupied. It could have to do with a tension between stressors and dispersal capacity, or could be a result of the vegetation community (density of redberry, buckwheat or other nectar plants). If following females and observing oviposition behavior does not shed light on habitat requirements, a more in-depth study of the vegetation community, its structure and other aspects may be warranted.



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## LITERATURE CITED

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- Berres ME, WR Engels and JAW. Kirsch. *in review*. A method for genotyping ostensibly dominant markers in AFLP fingerprints. *BMC Genomics*.
- Brattstrom O, S Akesson, and S Bensch. 2010. AFLP reveals cryptic population structure in migratory European red admirals (*Vanessa atalanta*). *Ecological Entomology*. 35: 248-252.
- Brown JW. 1991. Sensitive and declining butterfly species (Insecta: Lepidoptera) in San Diego County, California. Draft Report Prepared for Dudek and Associates.
- Coyle HM, G Shutler, L Tully, E Pagliaro, A Harper, T Palmbach, HC Lee. 2005. Validation of a DNA method for the individualization of plant evidence. National Institute of Justice. Grant # NIJ 2001-IJ-CX-K011.
- Deutschman DH, ME Berres, DA Marschalek and SL Strahm. 2010. Initial Evaluation of the Status of Hermes copper (*Lycaena hermes*) On Conserved Lands in San Diego County. Final Report for San Diego Association of Governments Contract: MOU # 5001442. 41 pages.
- Edwards WH. 1870. American Lepidoptera: *Chrysophanus hermes*. T. Am. Entomol. Soc. 3.
- Ehrlich, P. R., and S. E. Davidson. 1960. Techniques for capture-recapture studies of Lepidoptera populations. *Journal of the Lepidopterists' Society*. 14:227-229.
- Emmel TC and JF Emmel. 1973. The butterflies of southern California. Natural History Museum, Los Angeles County, Science Service 26: 1-148.
- Falush, D, M Stephens and J Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*. 164: 1567–1587.
- Fancy SG, JE Gross, and SL Carter. 2009. Monitoring the condition of natural resources in US national parks. *Environmental Monitoring and Assessment* 151(1-4): 161-174.
- Faulkner D. and M. Klein. 2004. San Diego's sensitive butterflies: a workshop focusing on 10 local species, San Diego, CA.
- Ferretti M. 2009. Quality Assurance in ecological monitoring-towards a unifying perspective. *Journal of Environmental Monitoring* 11(4): 726-729.
- Gloor GB, Preston CR, Johnson-Schlitz DM, Nassif NA, Phillis RW, Benz WK, Robertson HM, Engels WR. 1993. Type I repressors of P element mobility. *Genetics*. 135:81-95.
- Guillot, G., F. Mortier, A. Estoup. 2005. Geneland: A program for landscape genetics. *Molecular Ecology Notes*. 5:712-715.
- Hamm CA, D Aggarwal, DA Landis. 2010. Evaluating the impact of non-lethal DNA sampling on two butterflies, *Vanessa cardui* and *Satyrodes eurydice*. *Journal of Insect Conservation*. 14:11-18.
- INCA (2002). Insect Count Analyzer: A user-friendly program to analyze transect count data. The Urban Wildlands Group. [ <http://www.urbanwildlands.org/INCA/> ]
- Indsto JO, PH Weston, MA Clements, RJ Whelan. 2005. Highly sensitive DNA fingerprinting of orchid pollinaria remnants using AFLP. *Australian Systematic Botany*. 18: 207-213.
- Keyghobadi N, LA Crawford, SA Maxwell. 2009. Successful analysis of AFLPs from non-lethally sampled wing tissues in butterflies. *Conservation Genetics*. 10:2021-2024.

- Koscinski D, LA Crawford, HA Keller, N Keyghobadi. 2011. Effects of different methods of non-lethal tissue sampling on butterflies. *Ecological Entomology*. 36:301-308.
- Kull T., M Sammul, K Kull, K Lanno, K Tali, B Gruber, D Schmeller, and K Henle. 2008. Necessity and reality of monitoring threatened European vascular plants. *Biodiversity and Conservation* 17(14): 3383-3402.
- Legg C and L Nagy. 2006. Why most conservation monitoring is, but need not be, a waste of time. *Journal of Environmental Management* 78: 194-199.
- Marschalek DA. 2004. Factors influencing population viability of Hermes copper (*Lycaena hermes*). Master's Thesis. San Diego State University.
- Marschalek DA and DH Deutschman. 2008. Hermes copper (*Lycaena [Hermelycaena] hermes* : *Lycaenidae*): life history and population estimation of a rare butterfly. *Journal of Insect Conservation* 12(2): 97-105.
- Marschalek DA and DH Deutschman. 2009. "Larvae and Oviposition of Hermes Copper (Lepidoptera: Lycaenidae)." *Journal of Entomological Science* 44(4): 400-401.
- Marschalek DA and MW Klein. 2010. Distribution, ecology, and conservation of Hermes copper (*Lycaenidae: Lycena [Hermelycena] hermes*). *Journal of Insect Conservation*. 14:721-730.
- Marsh DM and PC Trenham. 2008. Current trends in plant and animal population monitoring. *Conservation Biology* 22(3): 647-655.
- McKelvey, K and D Pearson. 2001. Population estimation with sparse data: the role of estimators versus indices revisited." *Can J Zool* 79: 1754-1765.
- Medina RF, P Barbosa, M Christman, A Battisti. 2006. Number of individuals and molecular markers to use in genetic differentiation studies. *Molecular Ecology*. 6:1010-1013.
- Moller EM, Bahnweg G, Sanderman H, Geiger HH. 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Research*. 20:6115-6116.
- Morton AC. 1982. The effects of marking and capture on recapture frequencies of butterflies. *Oecologia*. 53:105-110.
- Ogden. 1996. Biological Monitoring Plan for the Multiple Species Conservation Program. San Diego, California.
- Ogden. 1998. Final Multiple Species Conservation Program. San Diego, California.
- Pollard E. 1988. Temperature, rainfall and butterfly numbers. *Journal of Applied Ecology*. 25:819-828.
- Reineke A, Karlovsky P, Zebitz CPW. 1998. Preparation and purification of DNA from insects for AFLP analysis. *Insect Molecular Biology*. 7:95-99.
- Roy DB, P Rothery, D Moss, E Pollard, and JA Thomas. 2001. Butterfly numbers and weather: Predicting historical trends in abundance and the future effects of climate change. *Journal of Animal Ecology*. 70:201-217.
- Schmeller DS, B Gruber, E Budrys, E Framsted, S Lengyel and K Henle. 2008. National responsibilities in European species conservation: A methodological review. *Conservation Biology* 22(3): 593-601.
- Singer MC, and P Wedlake. 1981. Capture does affect probability of recapture in a butterfly species. *Ecological Entomology*. 6:215-216.

- Starks, PT and JM Peters. 2002. Semi-nondestructive genetic sampling from live eusocial wasps, *Polistes dominulus* and *Polistes fuscatus*. *Insectes Soiaux*. 49(1): 20-22.
- Thorne F. 1963. The distribution of an endemic butterfly *Lycena hermes*. *J Res Lepid* 2: 143-150.
- United States Fish and Wildlife Service. 1989. Endangered and threatened wildlife and plants; Animal notice review. *Federal Register* 50 (CFR 17 54): 554-579.
- United States Fish and Wildlife Service. 1993. Endangered and threatened wildlife and plants; 90-day finding for a petition to list four California butterflies as endangered and continuation of status reviews. *Federal Register* 50 CFR (17 56): 58804-58836.
- United States Fish and Wildlife Service. 1996. Endangered and threatened species, plant and animal taxa; Proposed rule. *Federal Register* 50 CFR(17 61): 7596-7613.
- United States Fish and Wildlife Service. 2006. Endangered and threatened wildlife and plants; 90-day finding on a petition to list *Hermes copper* butterfly as endangered. *Federal Register* 50 CFR(17 71): 44966-44976.
- United States Fish and Wildlife Service. 2011. Endangered and threatened wildlife and plants; 12-Month Finding on a Petition To List *Hermes Copper* Butterfly as Endangered or Threatened. *Federal Register* 50 CFR(17): 20918-20939. <http://federalregister.gov/a/2011-9028>
- Vekemans X. 2002. AFLP-SURV version 1.0. Distributed by the author. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.
- Vekemans X, Beauwens T, Lemaire M, Roldan-Ruiz I. 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology*. 11:139-151.
- Vos P, Hogers R, Sleeker M, Reijans M, Lee T, Homes M, Freiters A, Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP: a new concept for DNA fingerprinting. *Nucleic Acids Research*. 23:4407-4414.
- Whitacre HW, BB Roper, and JL Kershner. 2007. A comparison of protocols and observer precision for measuring physical stream attributes. *Journal of the American Water Resources Association* 43(4): 923-937.
- White G, and K Burnham. 1999. Program MARK: survival estimation from populations of marked animals. *Bird Study* 46 (Supplement): 120–138.

## APPENDIX 1: 2011 HERMES COPPER LOCATIONS

Case	Date	Time	Site	Lat	Long	Elevation (ft)
176	5/31/2011	12:17:00 PM	Sycuan Peak	32.74853775	-116.8006888	2181
177	6/1/2011	11:57:00 AM	Sycuan Peak	32.7485826	-116.8006794	2194
178	6/2/2011	11:28:00 AM	Sycuan Peak	32.74754182	-116.8005118	2450
179	6/3/2011	10:35:58AM	Loveland Reservoir	32.78848	-116.79121	1363
180	6/3/2011	11:05:10AM	Loveland Reservoir	32.78957	-116.78601	1413
181	6/3/2011	12:35:48PM	Loveland Reservoir	32.78803	-116.77825	1451
182	6/4/2011	11:27:00 AM	Sycuan Peak	32.74858695	-116.8007235	2198
183	6/4/2011	11:37:00 AM	Sycuan Peak	32.74956353	-116.8003138	2253
184	6/4/2011	12:40:00 PM	Sycuan Peak	32.75152967	-116.8000792	2457
185	6/6/2011	11:06:00 AM	Sycuan Peak	32.74955012	-116.8003409	2270
186	6/6/2011	11:32:00 AM	Sycuan Peak	32.75272912	-116.8020135	2565
187	6/6/2011	12:20:00 PM	Sycuan Peak	32.74957837	-116.80035	2257
188	6/6/2011	12:35:00 PM	Sycuan Peak	32.74850716	-116.8006922	2198
189	6/7/2011	4:18:36PM	Meadowbrook	32.96234	-117.0707	624
190	6/8/2011	11:14:00 AM	Lawson Peak	32.71561867	-116.7070886	2273
191	6/8/2011	11:22:00 AM	Lawson Peak	32.71474485	-116.7103673	2483
192	6/8/2011	12:00:00 PM	Lawson Peak	32.71569855	-116.710576	2552
193	6/8/2011	12:35:16PM	McGinty Mountain	32.75686	-116.85461	1446
194	6/8/2011	2:08:52PM	McGinty Mountain	32.76838	-116.87135	917
195	6/8/2011	2:14:11PM	McGinty Mountain	32.76824	-116.87155	928
196	6/8/2011	2:26:52PM	McGinty Mountain	32.766	-116.87326	901
197	6/8/2011	2:29:36PM	McGinty Mountain	32.75904	-116.86797	1335
198	6/8/2011	2:37:32PM	McGinty Mountain	32.76451	-116.87403	848
199	6/8/2011	2:58:22PM	McGinty Mountain	32.76212	-116.87255	1098
200	6/8/2011	3:03:20PM	McGinty Mountain	32.76298	-116.87238	1000
201	6/8/2011	3:29:57PM	McGinty Mountain	32.76449	-116.87406	840
202	6/8/2011	3:37:56PM	McGinty Mountain	32.76539	-116.87406	870
203	6/8/2011	3:40:15PM	McGinty Mountain	32.76565	-116.87371	879
204	6/8/2011	3:56:18PM	McGinty Mountain	32.75725	-116.88298	1042
205	6/8/2011	4:18:25PM	McGinty Mountain	32.76783	-116.86629	1000
206	6/8/2011	4:29:12PM	McGinty Mountain	32.76691	-116.86336	1052
207	6/8/2011	5:20:35PM	McGinty Mountain	32.76145	-116.84974	948
208	6/8/2011	2:42:00 PM	Wright's Field	32.82130709	-116.7710392	1935

209	6/9/2011	11:37:00 AM	Sycuan Peak	32.75214926	-116.8019158	2536
210	6/9/2011	11:43:00 AM	Sycuan Peak	32.75287254	-116.8021137	2578
211	6/9/2011	12:33:00 PM	Sycuan Peak	32.75322977	-116.8047664	2709
212	6/9/2011	12:52:00 PM	Sycuan Peak	32.75199043	-116.8015096	2506
213	6/9/2011	1:08:00 PM	Sycuan Peak	32.75036383	-116.8004099	2355
214	6/9/2011	1:18:00 PM	Sycuan Peak	32.74957267	-116.800292	2253
215	6/9/2011	1:26:00 PM	Sycuan Peak	32.74887697	-116.8001169	2224
216	6/10/2011	1:06:51PM	Loveland Reservoir	32.7917	-116.78326	1472
217	6/10/2011	11:51:05AM	Loveland Reservoir	32.78842	-116.79125	1422
218	6/10/2011	11:57:03AM	Loveland Reservoir	32.78851	-116.79118	1413
219	6/10/2011	12:21:15PM	Loveland Reservoir	32.78975	-116.78788	1443
220	6/10/2011	12:30:26PM	Loveland Reservoir	32.79029	-116.78721	1506
221	6/10/2011	12:54:15PM	Loveland Reservoir	32.78959	-116.78365	1448
222	6/10/2011	2:41:40PM	Loveland Reservoir	32.79049	-116.77678	1441
223	6/13/2011	1:10:07PM	McGinty Mountain	32.74271	-116.86407	1555
224	6/13/2011	1:24:05PM	McGinty Mountain	32.74324	-116.86354	1565
225	6/13/2011	1:30:54PM	McGinty Mountain	32.74343	-116.86312	1574
226	6/13/2011	2:18:37PM	McGinty Mountain	32.74214	-116.8618	1407
227	6/13/2011	2:53:06PM	McGinty Mountain	32.73535	-116.86607	1368
228	6/13/2011	4:41:05PM	McGinty Mountain	32.7681	-116.86892	938
229	6/13/2011	4:50:42PM	McGinty Mountain	32.76845	-116.86762	981
230	6/13/2011	4:55:11PM	McGinty Mountain	32.76799	-116.86637	1002
231	6/13/2011	5:00:50PM	McGinty Mountain	32.76775	-116.8656	1021
232	6/13/2011	5:02:46PM	McGinty Mountain	32.76769	-116.86516	1032
233	6/13/2011	10:23:00 AM	Sycuan Peak	32.7474103	-116.7995556	2053
234	6/13/2011	10:41:00 AM	Sycuan Peak	32.74850305	-116.800644	2198
235	6/13/2011	10:56:00 AM	Sycuan Peak	32.74887756	-116.8001162	2201
236	6/13/2011	10:56:00 AM	Sycuan Peak	32.74887688	-116.8001159	2201
237	6/13/2011	11:07:00 AM	Sycuan Peak	32.74955037	-116.8003592	2270
238	6/13/2011	11:14:00 AM	Sycuan Peak	32.75040977	-116.8004532	2306
239	6/13/2011	11:47:00 AM	Sycuan Peak	32.75162816	-116.800701	2480
240	6/13/2011	11:53:00 AM	Sycuan Peak	32.75215262	-116.8018878	2536
241	6/13/2011	11:58:00 AM	Sycuan Peak	32.75285644	-116.8021194	2588
242	6/13/2011	12:06:00 PM	Sycuan Peak	32.75267355	-116.803015	2627
243	6/13/2011	12:14:00 PM	Sycuan Peak	32.75272728	-116.803272	2641
244	6/13/2011	12:33:00 PM	Sycuan Peak	32.7532312	-116.8047727	2716
245	6/13/2011	12:38:00 PM	Sycuan Peak	32.75373529	-116.8052336	2762
246	6/13/2011	1:27:00 PM	Sycuan Peak	32.74723579	-116.7999836	2034
247	6/13/2011	2:04:53PM	Wright's Field	32.82111	-116.76945	1934
248	6/14/2011	1:36:16PM	Elfin Forest	33.07491	-117.15936	445
249	6/15/2011	10:40:13AM	California Riding and Hiking Trail	32.79846	-116.75838	1507

250	6/15/2011	11:37:20AM	Los Montanas South	32.72712	-116.89996	630
251	6/15/2011	11:44:25AM	Los Montanas South	32.72696	-116.90012	664
252	6/15/2011	11:47:45AM	Los Montanas South	32.72688	-116.90029	664
253	6/15/2011	10:14:00 AM	Sycuan Peak	32.74722096	-116.8000281	2027
254	6/15/2011	10:23:00 AM	Sycuan Peak	32.74761767	-116.7995264	2096
255	6/15/2011	10:31:00 AM	Sycuan Peak	32.74819334	-116.800004	2152
256	6/15/2011	10:38:00 AM	Sycuan Peak	32.74847405	-116.8003286	2175
257	6/15/2011	10:43:00 AM	Sycuan Peak	32.74857405	-116.8006919	2185
258	6/15/2011	10:47:00 AM	Sycuan Peak	32.74884805	-116.8001152	2214
259	6/15/2011	10:53:00 AM	Sycuan Peak	32.74909163	-116.800132	2214
260	6/15/2011	10:57:00 AM	Sycuan Peak	32.74957149	-116.8003203	2260
261	6/15/2011	11:07:00 AM	Sycuan Peak	32.7508836	-116.7996133	2395
262	6/15/2011	11:23:00 AM	Sycuan Peak	32.75154602	-116.8005678	2477
263	6/15/2011	11:26:00 AM	Sycuan Peak	32.75162254	-116.8007145	2477
264	6/15/2011	11:34:00 AM	Sycuan Peak	32.75287287	-116.8021293	2578
265	6/15/2011	11:39:00 AM	Sycuan Peak	32.75273222	-116.8033458	2644
266	6/15/2011	11:51:00 AM	Sycuan Peak	32.75322927	-116.8047658	2716
267	6/15/2011	11:52:00 AM	Sycuan Peak	32.75319667	-116.8047546	2713
268	6/15/2011	11:56:00 AM	Sycuan Peak	32.75372079	-116.8052589	2759
269	6/15/2011	12:16:00 PM	Sycuan Peak	32.75299382	-116.8038345	2667
270	6/15/2011	12:33:00 PM	Sycuan Peak	32.74988037	-116.8003529	2306
271	6/15/2011	12:41:00 PM	Sycuan Peak	32.74937209	-116.8002313	2253
272	6/15/2011	12:44:00 PM	Sycuan Peak	32.74849224	-116.8006546	2191
273	6/15/2011	12:47:00 PM	Sycuan Peak	32.74770359	-116.7998811	2139
274	6/16/2011	9:46:00 AM	Lawson Peak	32.71336678	-116.7058669	2162
275	6/16/2011	9:46:00 AM	Lawson Peak	32.713419	-116.7058188	2158
276	6/16/2011	9:46:00 AM	Lawson Peak	32.71341808	-116.7058168	2158
277	6/16/2011	9:54:00 AM	Lawson Peak	32.71382486	-116.705678	2168
278	6/16/2011	9:54:00 AM	Lawson Peak	32.71382452	-116.705679	2168
279	6/16/2011	10:01:00 AM	Lawson Peak	32.71457981	-116.7057425	2214
280	6/16/2011	10:02:00 AM	Lawson Peak	32.71464469	-116.7058966	2214
281	6/16/2011	10:10:00 AM	Lawson Peak	32.71565395	-116.7070926	2293
282	6/16/2011	10:26:00 AM	Lawson Peak	32.71465927	-116.7098089	2522
283	6/16/2011	10:31:00 AM	Lawson Peak	32.7145457	-116.7098739	2490
284	6/16/2011	10:35:00 AM	Lawson Peak	32.71464913	-116.710298	2513
285	6/16/2011	10:35:00 AM	Lawson Peak	32.71465131	-116.7102958	2513
286	6/16/2011	10:41:00 AM	Lawson Peak	32.71470319	-116.7103199	2526
287	6/16/2011	10:45:00 AM	Lawson Peak	32.71571414	-116.7104652	2549
288	6/16/2011	10:57:00 AM	Lawson Peak	32.71732899	-116.7125274	2673
289	6/16/2011	1:02:14PM	McGinty Mountain	32.75698	-116.85509	1444
290	6/16/2011	1:05:27PM	McGinty Mountain	32.75672	-116.85539	1484

291	6/16/2011	1:10:45PM	McGinty Mountain	32.75633	-116.85546	1502
292	6/16/2011	1:14:32PM	McGinty Mountain	32.75599	-116.85562	1536
293	6/16/2011	1:36:38PM	McGinty Mountain	32.75314	-116.85812	1830
294	6/16/2011	1:54:14PM	McGinty Mountain	32.75537	-116.86151	1951
295	6/16/2011	12:07:22PM	McGinty Mountain	32.75898	-116.85108	1147
296	6/16/2011	12:16:17PM	McGinty Mountain	32.75903	-116.85154	1209
297	6/16/2011	12:36:39PM	McGinty Mountain	32.75747	-116.85438	1404
298	6/16/2011	12:51:28PM	McGinty Mountain	32.75694	-116.85445	1454
299	6/16/2011	12:57:09PM	McGinty Mountain	32.75691	-116.85467	1431
300	6/16/2011	2:13:31PM	McGinty Mountain	32.75701	-116.8644	1623
301	6/16/2011	2:18:30PM	McGinty Mountain	32.75727	-116.86442	1591
302	6/16/2011	2:25:44PM	McGinty Mountain	32.75815	-116.86537	1505
303	6/16/2011	2:32:38PM	McGinty Mountain	32.75757	-116.86578	1463
304	6/16/2011	2:38:14PM	McGinty Mountain	32.75752	-116.86601	1450
305	6/16/2011	2:44:26PM	McGinty Mountain	32.75749	-116.86675	1398
306	6/16/2011	2:50:29PM	McGinty Mountain	32.75798	-116.86725	1347
307	6/16/2011	4:13:25PM	McGinty Mountain	32.76412	-116.87437	817
308	6/16/2011	4:20:23PM	McGinty Mountain	32.7651	-116.87433	857
309	6/16/2011	4:29:32PM	McGinty Mountain	32.76653	-116.87262	917
310	6/16/2011	4:37:29PM	McGinty Mountain	32.76822	-116.87155	916
311	6/16/2011	4:45:36PM	McGinty Mountain	32.7683	-116.86923	926
312	6/16/2011	4:59:03PM	McGinty Mountain	32.76812	-116.86646	996
313	6/16/2011	5:01:09PM	McGinty Mountain	32.76787	-116.86612	999
314	6/16/2011	5:03:32PM	McGinty Mountain	32.76775	-116.86566	1010
315	6/16/2011	5:05:43PM	McGinty Mountain	32.76769	-116.86501	1021
316	6/16/2011	12:36:00 PM	Robert's Ranch N	32.82745513	-116.615452	3559
317	6/16/2011	12:38:00 PM	Robert's Ranch N	32.82759519	-116.6150312	3549
318	6/16/2011	12:45:00 PM	Robert's Ranch N	32.82785612	-116.6144034	3585
319	6/16/2011	12:45:00 PM	Robert's Ranch N	32.82785671	-116.6144072	3585
320	6/16/2011	1:00:00 PM	Robert's Ranch N	32.82747248	-116.6149136	3592
321	6/17/2011	9:56:00 AM	Loveland Reservoir	32.79176063	-116.7832514	1446
322	6/17/2011	10:17:00 AM	Loveland Reservoir	32.79022808	-116.7873124	1433
323	6/17/2011	10:24:00 AM	Loveland Reservoir	32.78977102	-116.7877936	1443
324	6/17/2011	10:37:00 AM	Loveland Reservoir	32.78965786	-116.7901041	1423
325	6/17/2011	10:47:00 AM	Loveland Reservoir	32.78847442	-116.7911597	1400
326	6/17/2011	11:14:00 AM	Loveland Reservoir	32.79024032	-116.7873059	1440
327	6/17/2011	11:19:00 AM	Loveland Reservoir	32.78971972	-116.786183	1427
328	6/17/2011	11:19:00 AM	Loveland Reservoir	32.78972383	-116.7861769	1427
329	6/17/2011	11:28:00 AM	Loveland Reservoir	32.78943012	-116.7849587	1420
330	6/17/2011	11:38:00 AM	Loveland Reservoir	32.79110868	-116.7831383	1433
331	6/17/2011	11:44:00 AM	Loveland Reservoir	32.79224007	-116.7829106	1479

332	6/17/2011	12:08:41PM	Loveland Reservoir	32.79326	-116.7761	1538
333	6/17/2011	12:01:46PM	McGinty Mountain	32.76185	-116.8855	429
334	6/17/2011	12:06:26PM	McGinty Mountain	32.76207	-116.88465	448
335	6/17/2011	12:50:00 PM	Sycuan Peak	32.74691803	-116.7994954	2024
336	6/17/2011	12:55:00 PM	Sycuan Peak	32.74726731	-116.8000211	2040
337	6/17/2011	12:59:00 PM	Sycuan Peak	32.74768774	-116.7995312	2086
338	6/17/2011	1:01:00 PM	Sycuan Peak	32.74775513	-116.7998587	2103
339	6/17/2011	1:04:00 PM	Sycuan Peak	32.74816853	-116.7999346	2129
340	6/17/2011	1:13:00 PM	Sycuan Peak	32.74883774	-116.8001437	2227
341	6/17/2011	1:18:00 PM	Sycuan Peak	32.74955213	-116.8003383	2253
342	6/17/2011	1:25:00 PM	Sycuan Peak	32.75035311	-116.8003979	2349
343	6/17/2011	1:32:00 PM	Sycuan Peak	32.75153344	-116.800123	2463
344	6/17/2011	1:35:00 PM	Sycuan Peak	32.75159916	-116.8006637	2470
345	6/17/2011	1:38:00 PM	Sycuan Peak	32.75200023	-116.8015303	2500
346	6/17/2011	1:45:00 PM	Sycuan Peak	32.75264321	-116.8027896	2618
347	6/17/2011	1:50:00 PM	Sycuan Peak	32.75298947	-116.8038402	2660
348	6/17/2011	1:54:00 PM	Sycuan Peak	32.75324964	-116.8047552	2713
349	6/17/2011	1:54:00 PM	Sycuan Peak	32.7532452	-116.8047577	2716
350	6/17/2011	2:00:00 PM	Sycuan Peak	32.75374602	-116.8052757	2759
351	6/17/2011	2:07:00 PM	Sycuan Peak	32.75420065	-116.8055588	2791
352	6/17/2011	2:20:00 PM	Sycuan Peak	32.75292082	-116.8021156	2588
353	6/17/2011	2:22:00 PM	Sycuan Peak	32.7528743	-116.8021079	2582
354	6/17/2011	2:36:00 PM	Sycuan Peak	32.74883791	-116.8000817	2221
355	6/20/2011		Elfin Forest	33.07488	-117.1593	455
356	6/20/2011		Los Montanas North	32.73125	-116.88158	904
357	6/20/2011		Los Montanas South	32.72717	-116.9	654
358	6/20/2011		Los Montanas South	32.72684	-116.90012	679
359	6/20/2011	9:10:00 AM	Sycuan Peak	32.74686632	-116.7994485	2057
360	6/20/2011	9:13:00 AM	Sycuan Peak	32.7472456	-116.7999888	2060
361	6/20/2011	9:21:00 AM	Sycuan Peak	32.7476103	-116.7994901	2093
362	6/20/2011	9:31:00 AM	Sycuan Peak	32.74857539	-116.8006929	2194
363	6/20/2011	9:35:00 AM	Sycuan Peak	32.7488421	-116.80011	2217
364	6/20/2011	9:49:00 AM	Sycuan Peak	32.75151308	-116.8001464	2483
365	6/20/2011	9:54:00 AM	Sycuan Peak	32.75201038	-116.8015224	2513
366	6/20/2011	10:01:00 AM	Sycuan Peak	32.75287136	-116.8021238	2575
367	6/20/2011	10:03:00 AM	Sycuan Peak	32.75281697	-116.8023123	2591
368	6/20/2011	10:12:00 AM	Sycuan Peak	32.75299508	-116.8038525	2664
369	6/20/2011	10:18:00 AM	Sycuan Peak	32.75319734	-116.8048169	2729
370	6/20/2011	10:21:00 AM	Sycuan Peak	32.75373168	-116.8052432	2749
371	6/20/2011	10:26:00 AM	Sycuan Peak	32.75434398	-116.805824	2788
372	6/20/2011	10:47:00 AM	Sycuan Peak	32.75297966	-116.8038476	2667



373	6/20/2011	11:00:00 AM	Sycuan Peak	32.75265981	-116.8020263	2562
374	6/20/2011	11:14:00 AM	Sycuan Peak	32.74954593	-116.8003348	2263
375	6/20/2011	11:21:00 AM	Sycuan Peak	32.7488587	-116.8001146	2224
376	6/20/2011	11:31:00 AM	Sycuan Peak	32.74761222	-116.7994923	2099
377	6/20/2011	10:39:42AM	Wright's Field	32.82182	-116.77033	1950
378	6/20/2011	10:40:29AM	Wright's Field	32.82182	-116.77032	1903
379	6/20/2011	10:49:18AM	Wright's Field	32.82204	-116.77009	1870
380	6/21/2011	1:41:20PM	McGinty Mountain	32.76316	-116.87189	1040
381	6/21/2011	1:47:24PM	McGinty Mountain	32.76302	-116.87249	999
382	6/21/2011	1:54:47PM	McGinty Mountain	32.76413	-116.8744	848
383	6/21/2011	2:19:58PM	McGinty Mountain	32.76648	-116.87266	965
384	6/21/2011	3:48:03PM	McGinty Mountain	32.76206	-116.88465	400
385	6/21/2011	10:39:00 AM	Robert's Ranch N	32.82757415	-116.6149911	3549
386	6/21/2011	10:45:00 AM	Robert's Ranch N	32.82770734	-116.6144102	3585
387	6/21/2011	10:51:00 AM	Robert's Ranch N	32.82784254	-116.6144117	3592
388	6/21/2011	10:55:00 AM	Robert's Ranch N	32.82767901	-116.6144288	3582
389	6/21/2011	9:32:00 AM	Wildwood Glen	32.8419668	-116.639976	3320
390	6/22/2011	10:11:58AM	Lopez Canyon	32.9137	-117.17621	194
391	6/22/2011	10:25:14AM	Lopez Canyon	32.91412	-117.17712	209
392	6/22/2011	10:33:59AM	Lopez Canyon	32.9138	-117.1773	201
393	6/22/2011	10:48:28AM	Lopez Canyon	32.91341	-117.17837	193
394	6/22/2011	10:58:02AM	Lopez Canyon	32.91327	-117.17909	182
395	6/22/2011	9:11:00 AM	Sycuan Peak	32.74690169	-116.7994853	2017
396	6/22/2011	9:20:00 AM	Sycuan Peak	32.74761893	-116.7995182	2096
397	6/22/2011	9:23:00 AM	Sycuan Peak	32.74775061	-116.7998763	2109
398	6/22/2011	9:35:00 AM	Sycuan Peak	32.74858763	-116.8006831	2188
399	6/22/2011	9:43:00 AM	Sycuan Peak	32.74886121	-116.8000877	2221
400	6/22/2011	9:56:00 AM	Sycuan Peak	32.7501874	-116.8004483	2322
401	6/22/2011	10:03:00 AM	Sycuan Peak	32.75025277	-116.8004899	2312
402	6/22/2011	10:06:00 AM	Sycuan Peak	32.75032771	-116.8004013	2345
403	6/22/2011	10:22:00 AM	Sycuan Peak	32.75151299	-116.8001258	2477
404	6/22/2011	10:32:00 AM	Sycuan Peak	32.75280288	-116.8023148	2582
405	6/22/2011	10:37:00 AM	Sycuan Peak	32.75274991	-116.8033916	2654
406	6/22/2011	10:41:00 AM	Sycuan Peak	32.75297421	-116.8038551	2664
407	6/22/2011	10:56:00 AM	Sycuan Peak	32.75374526	-116.8052625	2759
408	6/22/2011	11:01:00 AM	Sycuan Peak	32.75409806	-116.8055665	2785
409	6/22/2011	11:08:00 AM	Sycuan Peak	32.7544112	-116.8060034	2798
410	6/22/2011	11:25:00 AM	Sycuan Peak	32.75372498	-116.8052647	2746
411	6/22/2011	11:31:00 AM	Sycuan Peak	32.7528753	-116.8034532	2664
412	6/22/2011	11:36:00 AM	Sycuan Peak	32.75282719	-116.802287	2591
413	6/22/2011	11:38:00 AM	Sycuan Peak	32.75266425	-116.8020001	2559

414	6/22/2011	11:43:00 AM	Sycuan Peak	32.75198003	-116.8015449	2516
415	6/22/2011	11:46:00 AM	Sycuan Peak	32.7517507	-116.8010123	2490
416	6/22/2011	11:49:00 AM	Sycuan Peak	32.75168826	-116.8008142	2480
417	6/22/2011	12:06:00 PM	Sycuan Peak	32.74961114	-116.8003762	2273
418	6/22/2011	12:06:00 PM	Sycuan Peak	32.74960963	-116.800377	2276
419	6/22/2011	12:11:00 PM	Sycuan Peak	32.74894754	-116.8001275	2214
420	6/22/2011	12:16:00 PM	Sycuan Peak	32.74885903	-116.8001118	2211
421	6/22/2011	12:23:00 PM	Sycuan Peak	32.74819208	-116.799989	2148
422	6/23/2011	10:53:00AM	California Riding and Hiking Trail	32.79973	-116.76133	1459
423	6/23/2011	9:26:00 AM	Lawson Peak	32.71539546	-116.7104336	2529
424	6/23/2011	9:37:00 AM	Lawson Peak	32.71468534	-116.7102429	2539
425	6/23/2011	9:54:00 AM	Lawson Peak	32.7143395	-116.7055402	2191
426	6/23/2011	10:00:00 AM	Lawson Peak	32.71393156	-116.7055929	2178
427	6/23/2011	10:05:00 AM	Lawson Peak	32.71361531	-116.7057857	2162
428	6/23/2011	10:06:11AM	Loveland Extension	32.79237	-116.74479	1340
429	6/24/2011	10:04:14AM	Loveland Reservoir	32.79047	-116.77684	1436
430	6/24/2011	11:08:09AM	Loveland Reservoir	32.79173	-116.78322	1445
431	6/24/2011	11:33:46AM	Loveland Reservoir	32.78923	-116.78477	1413
432	6/24/2011	11:41:33AM	Loveland Reservoir	32.79008	-116.78651	1432
433	6/24/2011	11:47:26AM	Loveland Reservoir	32.79035	-116.787	1437
434	6/24/2011	1:16:27PM	Sycuan Peak	32.75283	-116.80233	2582
435	6/24/2011	1:21:57PM	Sycuan Peak	32.75201	-116.80151	2499
436	6/24/2011	1:29:44PM	Sycuan Peak	32.75178	-116.80102	2482
437	6/24/2011	1:57:03PM	Sycuan Peak	32.74688	-116.79947	2029
438	6/24/2011	11:14:58AM	Sycuan Peak	32.74778	-116.79983	2080
439	6/24/2011	11:22:42AM	Sycuan Peak	32.74795	-116.79979	2099
440	6/24/2011	11:32:32AM	Sycuan Peak	32.74862	-116.80067	2155
441	6/24/2011	11:35:24AM	Sycuan Peak	32.74859	-116.80068	2159
442	6/24/2011	11:40:53AM	Sycuan Peak	32.7489	-116.80009	2180
443	6/24/2011	11:51:44AM	Sycuan Peak	32.75009	-116.80045	2286
444	6/24/2011	12:07:34PM	Sycuan Peak	32.7504	-116.80038	2323
445	6/24/2011	12:17:56PM	Sycuan Peak	32.75079	-116.79969	2367
446	6/24/2011	12:33:15PM	Sycuan Peak	32.75283	-116.80231	2568
447	6/24/2011	12:33:18PM	Sycuan Peak	32.75283	-116.80231	2563
448	6/27/2011	1:18:50PM	Sycuan Peak	32.74764	-116.79953	2115
449	6/27/2011	11:09:33AM	Sycuan Peak	32.74689	-116.79946	1996
450	6/27/2011	11:20:39AM	Sycuan Peak	32.74779	-116.79987	2095
451	6/27/2011	11:30:35AM	Sycuan Peak	32.7486	-116.80069	2173
452	6/27/2011	11:41:00AM	Sycuan Peak	32.74888	-116.80011	2194
453	6/27/2011	11:51:32AM	Sycuan Peak	32.74961	-116.80036	2248
454	6/27/2011	11:56:33AM	Sycuan Peak	32.74959	-116.80033	2248

455	6/27/2011	12:13:24PM	Sycuan Peak	32.75205	-116.80153	2483
456	6/27/2011	12:48:27PM	Sycuan Peak	32.75303	-116.80389	2659
457	6/29/2011	2:49:37PM	California Riding and Hiking Trail	32.79964	-116.76131	1484
458	6/29/2011	12:08:23PM	Loveland Extension	32.79062	-116.74341	1390
459	6/29/2011	10:15:21AM	McGinty Mountain	32.76183	-116.8854	407
460	6/29/2011	10:16:19AM	Robert's Ranch N	32.82779	-116.61469	3590
461	6/29/2011	10:27:02AM	Robert's Ranch N	32.82759	-116.61506	3559
462	6/29/2011	10:35:34AM	Robert's Ranch N	32.82699	-116.61555	3541
463	6/29/2011	9:07:44AM	Robert's Ranch N	32.82847	-116.61769	3412
464	6/29/2011	9:23:19AM	Robert's Ranch N	32.82725	-116.61635	3510
465	6/29/2011	9:40:13AM	Robert's Ranch N	32.82749	-116.6147	3564
466	6/29/2011	9:46:52AM	Robert's Ranch N	32.82771	-116.6144	3584
467	6/29/2011	9:51:48AM	Robert's Ranch N	32.82777	-116.61439	3592
468	6/29/2011	9:58:33AM	Robert's Ranch N	32.82789	-116.6144	3595
469	6/29/2011	1:06:46PM	Sycuan Peak	32.74851	-116.80064	2196
470	6/29/2011	1:14:35PM	Sycuan Peak	32.74772	-116.79983	2125
471	6/29/2011	1:14:42PM	Sycuan Peak	32.74772	-116.79983	2127
472	6/29/2011	1:33:58PM	Sycuan Peak	32.7469	-116.79947	2039
473	6/29/2011	11:20:52AM	Sycuan Peak	32.74886	-116.80007	2189
474	6/29/2011	11:26:17AM	Sycuan Peak	32.7496	-116.80032	2239
475	6/29/2011	11:28:33AM	Sycuan Peak	32.74963	-116.80035	2243
476	6/29/2011	11:29:44AM	Sycuan Peak	32.74956	-116.80032	2240
477	6/29/2011	12:03:42PM	Sycuan Peak	32.75304	-116.80387	2647
478	6/29/2011	12:35:38PM	Sycuan Peak	32.75205	-116.80168	2515
479	6/29/2011	12:40:46PM	Wildwood Glen	32.84093	-116.65095	3197
480	6/29/2011	12:50:37PM	Wildwood Glen	32.84081	-116.65106	3191
481	6/29/2011	12:54:25PM	Wildwood Glen	32.84099	-116.65087	3198
482	6/29/2011	2:06:32PM	Wright's Field	32.82108	-116.7711	1970
483	6/30/2011	10:03:52AM	McGinty Mountain	32.75674	-116.85534	1473
484	6/30/2011	10:08:10AM	McGinty Mountain	32.75648	-116.85545	1513
485	6/30/2011	10:18:59AM	McGinty Mountain	32.75578	-116.8558	1522
486	6/30/2011	10:26:29AM	McGinty Mountain	32.75572	-116.85584	1535
487	6/30/2011	10:38:26AM	McGinty Mountain	32.75448	-116.85672	1609
488	6/30/2011	11:03:36AM	McGinty Mountain	32.75191	-116.8571	1757
489	6/30/2011	11:14:26AM	McGinty Mountain	32.75308	-116.85813	1834
490	6/30/2011	11:19:16AM	McGinty Mountain	32.75381	-116.85838	1871
491	6/30/2011	11:32:46AM	McGinty Mountain	32.75557	-116.86025	1976
492	6/30/2011	11:59:35AM	McGinty Mountain	32.75557	-116.86025	1984
493	6/30/2011	12:10:51PM	McGinty Mountain	32.75612	-116.86227	1862
494	6/30/2011	12:18:06PM	McGinty Mountain	32.75584	-116.86296	1843
495	6/30/2011	12:25:29PM	McGinty Mountain	32.75665	-116.86293	1741

496	6/30/2011	12:35:36PM	McGinty Mountain	32.75692	-116.86286	1730
497	6/30/2011	12:44:31PM	McGinty Mountain	32.75736	-116.86447	1584
498	6/30/2011	12:53:18PM	McGinty Mountain	32.75755	-116.86578	1456
499	6/30/2011	2:43:02PM	McGinty Mountain	32.76821	-116.87165	929
500	6/30/2011	9:45:23AM	McGinty Mountain	32.76784	-116.86607	1010
501	6/30/2011	9:52:52AM	McGinty Mountain	32.76676	-116.86298	1058
502	7/1/2011	10:55:35AM	California Riding and Hiking Trail	32.74688	-116.79943	2000
503	7/1/2011	10:59:55AM	California Riding and Hiking Trail	32.74739	-116.79963	2042
504	7/1/2011	10:21:26AM	Loveland Reservoir	32.79033	-116.78723	1450
505	7/1/2011	11:05:19AM	Sycuan Peak	32.74781	-116.79987	2095
506	7/1/2011	11:12:32AM	Sycuan Peak	32.74857	-116.80065	2166
507	7/1/2011	11:49:39AM	Sycuan Peak	32.7528	-116.8034	2624
508	7/1/2011	11:59:38AM	Sycuan Peak	32.75333	-116.80447	2698
509	7/1/2011	12:39:54PM	Sycuan Peak	32.74957	-116.80034	2278
510	7/6/2011	9:19:23AM	Robert's Ranch N	32.82703	-116.61552	3541
511	7/6/2011	9:29:09AM	Robert's Ranch N	32.82727	-116.6164	3510
512			McGinty Mountain	32.74337	-116.86312	1567
513			McGinty Mountain	32.73732	-116.86519	1352
514			McGinty Mountain	32.73563	-116.86571	1361
515			McGinty Mountain	32.75994	-116.85163	1133
516			McGinty Mountain	32.75995	-116.85163	1136
517			McGinty Mountain	32.75894	-116.85094	1152
518			McGinty Mountain	32.7569	-116.85463	1428
519			McGinty Mountain	32.75677	-116.85524	1483
520			McGinty Mountain	32.75668	-116.85542	1499
521			McGinty Mountain	32.75642	-116.85547	1504
522			McGinty Mountain	32.75618	-116.85553	1523
523			McGinty Mountain	32.75616	-116.85559	1525
524			McGinty Mountain	32.75546	-116.85612	1569
525			McGinty Mountain	32.75536	-116.85619	1579
526			McGinty Mountain	32.75537	-116.86151	1938
527			McGinty Mountain	32.75683	-116.86273	1720
528			McGinty Mountain	32.75701	-116.8644	1610
529			McGinty Mountain	32.7582	-116.86537	1510

Note: Summary counts for Hermes copper presented in the report above may vary slightly from this table. This is because these data are collected differently using a GPS and occasionally an observation will get marked twice, or an observation with multiple Hermes will only be GPSed once.

Cases are numbered starting off from last year's observations and therefore start at 176.

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## APPENDIX 2: BRIEF FIELD NOTES

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- Spring Azure and Northern Cloudywing Skipper skipper need to be added to the butterfly list.
- Hermes was seen mating within 20 minutes of having a leg removed.
- 2 mating events were witness this year.