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## Effect of Exposure to Gypsum Dust on Survival of Behr's Metalmark

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July 21, 2015

### Introduction

Lange's metalmark butterfly (*Apodemia mormo langei*), an endemic species to the Antioch dunes, is critically endangered and at risk of extinction. One of the threats to this species is exposure to gypsum dust that drifts into its habitat at the Antioch Dunes National Wildlife Refuge from a gypsum plant immediately adjacent to it. This has long been suspected as a hazard to the species, either in the adult or larval stage because of the potential for gypsum to cause physical harm to the species externally through abrasion or similarly to harm larvae internally after consumption. A 2013 workshop included gypsum dust on a list of threat rankings and confirmed the desire for further research (Richmond et al. 2015).

Although a captive propagation program for Lange's metalmark is in place, the number of individuals is too small to allow experiments that might harm the butterflies (Johnson et al. 2007). Therefore we used the common Behr's metalmark (*Apodemia virgulti*), a related species, as a surrogate to undertake a pilot study on the impact of gypsum dust on both adults and larvae. Behr's metalmark was subsequently used as a surrogate for Lange's metalmark in other toxicity research (Stark et al. 2012). This experiment was undertaken in 2007 and the data are analyzed here for the first time.

### Methods

Behr's metalmarks were collected in the wild from four locations in Ventura County, California, near Moorpark College. Adults were then confined in boxes and provided California buckwheat buckwheat (*Eriogonum fasciculatum*) and *ad lib* honey/water solution. Due to the difficulty in distinguishing between the varieties of this buckwheat, a more precise identification was not made. All of the plant material that was used originated from a backyard garden. No pesticides or herbicides of any kind are used on the property. The plants were of two different origins. Some were grown from seed collected from a local site along the Los Robles Trail, in Westlake (less than 2 miles from where some of the adult metalmarks were collected). The other plants

were purchased from Matilija Nursery in Moorpark, in 2002. The geographic origin of this nursery stock is not known. The plants from these two sources exhibit different morphologies. The stock from Westlake has rather leathery, yellow-green, and sparsely-haired leaves. The Matilija Nursery stock displays slightly smaller and more rounded leaves, which are darker green and more densely haired. Larvae were maintained with ample foodplant and transferred between containers when necessary.

### *Gypsum Treatments*

Adult butterflies were treated with gypsum every week. All treatments consisted of hand-sprinkling the adults with the dust. Egg/larval treatments initially took place on the Wednesday schedule. But because the powder was observed to not blow away or otherwise disperse from week to week, treatment frequency was reduced.

### *Egg, and Larvae Surveys*

The number of eggs laid by each female was estimated by visual inspection, as was the number of larvae hatching and growing from each female. These surveys were all undertaken by the same person but do not represent exhaustive searches because too much disturbance harms the larvae (Johnson et al. 2007).

Survival of adults was calculated using the software JMP 11 Pro (SAS, Cary, NC). Differences between treatments for survival was tested with the nonparametric Cox-Mantel log-rank test. Differences in number of eggs and larvae by treatment was calculated with JMP.

## **Results**

Twenty-one butterflies were captured for the experiment and one captive reared adult from a previous rearing effort with the species was used. All the adult butterflies and 1<sup>st</sup>-instar larvae were exposed exclusively to the Westlake plant stock. During the larval relocation of December 7<sup>th</sup>, all larvae were transferred to a mixture of the two plant stocks. The proportion of the two stocks in each larval container was standardized: eight stems of Matilija Nursery stock, and four stems of Westlake stock. After this transfer, definite larval consumption of the Matilija Nursery stock plant material was noted.

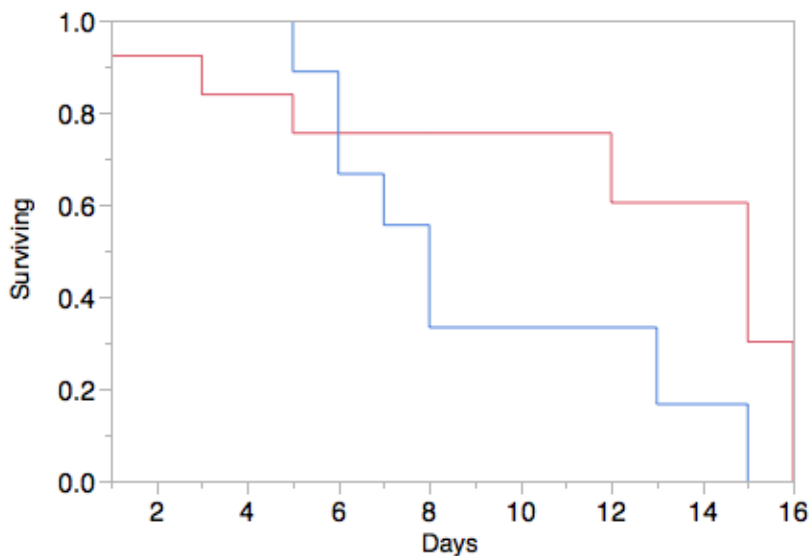
The adults were dusted with gypsum on September 26, October 3, October 10, and October 16, 2007. None of the butterflies took kindly to this procedure. "Irritated" fluttering was the rule. The powder was observed to stick poorly to the butterflies. In particular, it would quite readily slide off the wings. The powder would persist only when placed on the fuzzier bodies of the adults. Even so, well before the next treatment took place, virtually all powder had fallen away.

The larvae were treated on October 31, November 7, and December 12, 2007. The October and November treatments were accomplished via the hand-sprinkling method, just as with the adults. For the final treatment, in December, a sieve was used to apply the gypsum. Use of this sieve permitted a more uniform application of the powder.

A group of adults was released on October 22, 2007 when site had to be evacuated to avoid a wildfire. Following protocols, the butterflies that were not endangered were released, but the eggs and larvae were maintained and not damaged. The adults that were released were then censored from the analysis.

#### *Adult Survival*

Females lived slightly longer ( $10.25 \pm 2.04$  S.E. days;  $n=6$ ) than males ( $10.15 \pm 1.34$  S.E. days;  $n=9$ ). With the small sample size, this difference was not significant (Log-Rank  $\chi^2=0.85$ ;  $p<0.35$ ). Across both sexes, those exposed to gypsum lived  $9.11 \pm 1.29$  S.E. ( $n=8$ ) days after capture while those not exposed lived  $11.93 \pm 1.66$  S.E. ( $n=7$ ) days (Log-Rank  $\chi^2=3.09$ ;  $p<0.07$ ; Figure 1).



**Figure 1. Minimum longevity of wild-caught Behr's metalmarks either exposed (blue) or not exposed (red) to gypsum dust.**

#### *Egg and Larval Production*

The three females treated with gypsum produced 7, 18, and 24 eggs. Five control females laid 0, 12, 50, 60, and 96 eggs. Although the average number of control females was higher (43.8 eggs) than for treated females (16.3 eggs) the small sample size precludes statistical inference.

Similarly, on average more larvae were documented in the control containers (31.4) than in the treatment containers (17.3), but the experiment had to be terminated to provide these larvae to the Fish and Wildlife Service for a study on the effects of herbicides (Stark et al. 2012).

#### **Discussion**

The study was obviously hampered by the evacuation of the rearing facility and resulting loss of statistical power. Furthermore the age of specimens at capture was unknown, but this bias would be equally distributed across both treatments.

Even with the small sample size, the results suggest a negative effect of gypsum dust on adult longevity ( $p < 0.07$ ;  $n = 15$ ). The number of eggs and number of larvae are both in the direction that also suggest a negative effect of gypsum dust. A larger sample size would be necessary to further pursue the possible association between gypsum treatment and number of eggs and larvae produced. Gypsum dust may affect larval survival of the endangered Lange's metalmark and a surrogate species approach to investigating these effects is feasible and should be replicated with a larger sample size. Gypsum dust in a clay diluent has been documented as being lethal to termites (Wagner and Ebeling 1959), so the possibility for inadvertent impacts from gypsum dust on butterflies and larvae merits further investigation.

### **Literature Cited**

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