# EVIDENCE OF POLLEN DIGESTION AT NOCTURNAL AGGREGATIONS OF *HELICONIUS SARA* IN COSTA RICA (LEPIDOPTERA: NYMPHALIDAE)

**Christian Salcedo**
Dept. of Entomology and Nematology & McGuire Center for Lepidoptera and Biodiversity, University of Florida, Gainesville, FL 32611; salcedo@ufl.edu

**Abstract-** Several *Heliconius* butterflies species are known to rely widely on pollen supplied by flowers they visit on a daily basis. Pollen feeding provides a regular supply of amino acids and key nutrients, which are essential for fecundity, *de novo* synthesis of cyanide glycosides, and increased lifespan. This adaptation has been documented as a diurnal habit that occurs in males and females. Observations in *H. sara* revealed that only females arrive with pollen loads at dusk to roosting sites in Panama and Costa Rica. Further analysis of pollen samples shows that pollen collected by these females is being digested overnight.

**Key words:** *Heliconius*, nocturnal aggregation, pollen feeding

## INTRODUCTION

Many holometabolous insects have developed considerably different ecological and nutritional requirements in the larval and adult stages. For Lepidoptera, feeding on plant tissue is restricted to the larval stage, whereas the adult stage feeds primarily on plant-produced food supplements such as nectar, sap, rotten-fruit, dung, and pollen. An interesting example to study these complex multi-trophic level interactions is mimetic butterflies from the genus *Heliconius* (Nymphalidae). This genus is widely spread in the subtropical and tropical forests of America and has been extensively studied due to its ease of breeding and outstanding evolutionary adaptations. Some species in this genus present the habit of diurnal pollen feeding as adults (Gilbert 1972). Pollen-feeding genera include the monotypic *Laparus*, and *Heliconius* with 38 species, which is a significant representation of the New World heliconiine diversity (Gilbert 1991). *Heliconius* are unique in their systematic exploitation of pollen. During the day, adults spend long periods collecting pollen and occupy home ranges based largely on a network of pollen plants (Gilbert 1991). Pollen collecting involves inserting the proboscis in the flower with particular movements (Penz and Krenn 2000) to assure the adhesion of pollen grains (Krenn and Penz 1998) whereas pollen digestion occurs immediately afterwards when the pollen makes contact with the saliva to dissolve out amino acids (Eberhard *et al.* 2007, Eberhard *et al.* 2009). Pollen feeding provides a regular supply of amino acids to adult *Heliconius* butterflies, which is essential for adult fitness (Dunlap-Pianka *et al.* 1977, Brown *et al.* 1991). For example, *H. charithonia* in a free-flying greenhouse colony with access to pollen lived for an average of 35 days, with a maximum of 105 days which is a similar lifespan to that recorded in the field (Boggs 1979). Conversely, captive *H. charithonia* fed only on nectar or sugar water solutions lived 10-12 d on average (pers. obs). Oogenesis is also strongly influenced with marked decreases in oviposition rates when pollen is absent in the diet (O'Brien *et al.* 2003). Similarly, lifetime fecundity (number of eggs throughout the lifespan) drops drastically without the pollen nutritional complement (Dunlap-Pianka *et al.* 1977). Further, essential amino acids transfer from pollen to butterfly eggs has been demonstrated in *H. charithonia* using stable isotope variation (O'Brien *et al.* 2003).

Several *Heliconius* species form nocturnal aggregations in sites with a particular architecture. Roosts are usually formed under relatively dense vegetation mats where dry vines or branches provide a perch for the night. Despite some evidence on the ecological and behavioral mechanisms that may cause the expression of this behavior (unpub. data), its adaptive significance is yet unknown. Recent field observations at *H. sara* and *H. charithonia* roosting sites in Panama, Costa Rica, and Honduras showed that individuals arrive at roost sites with pollen loads at dusk. Pollen loads from butterflies at *H. sara* roost sites in Costa Rica were collected and analyzed for evidence of digestion. Nocturnal pollen digestion may be an adaptive strategy that maximizes pollen feeding by providing key nutritional resources to the butterflies while resting or sleeping. In addition, digesting pollen at roost sites may be beneficial and perhaps more efficient due to the fact that they offer particular characteristics such as protection from wind, rain, buffered humidity, and are almost free from predation (unpub. data). The present work shows evidence for nocturnal pollen digestion at *H. sara* roost sites in Costa Rica.

## MATERIALS AND METHODS

**Study Organisms and field sites:** Pollen samples were collected in La Selva field station, Organization for Tropical Studies, in Costa Rica. La Selva is an excellent site where *Heliconius sara* populations are stable and roost sites are accessible. *H. sara* has a widespread neotropical distribution from South Mexico to South Brazil (Holzinger and Holzinger 1994). It expresses nocturnal communal roosting at heights ranging from 1.5 m to 4 m (Mallet and Gilbert 1995).

La Selva was visited during April 2009, at the end of the dry season. The field station is located at the confluence of two major rivers in the Caribbean lowlands of northern Costa Rica. The area comprises 1,600 ha of tropical wet forests and disturbed lands.

**Roost finding:** Roost site architecture is a key visual aid when searching for roost sites in the field. *Heliconius* butterflies prefer shaded areas with plenty of thin dry vines and branches under relatively dense vegetation mats. These sites are often located near disturbed areas, forest edges, and trails. In order to identify potential roost sites, a preliminary search was done by exploring the area looking for sites with suitable characteristics. Roosting aggregations were found by hiking through La Selva trails and forest edges from 1600 to 1730 h; when a butterfly was spotted, it was followed until it arrived to the roost site. After dark, searches were also done taking into account the pre-selected sites found in the preliminary search.

**Collection and analysis of pollen samples:** *H. sara* individuals bearing pollen loads in their proboscises were captured when they were arriving at the roost sites at 1630-

1740 h and after they perched at 2000-2200 h. Pollen loads were removed from the proboscis using an insect pin and then the butterflies were released immediately. Microscope slides of pollen loads were prepared in the field. Pollen grains were directly washed off from the insect pin in small drops of glycerin onto glass slides. The slides were covered with cover glasses and sealed with nail enamel (Krenn *et al.* 2009). The samples were observed under a compound light microscope with a digital camera attached (Olympus BX60 and Hitachi KP-D50). At least 20 different photographs were taken for each slide. The most abundant pollen grains (90% or more on each sample) belonged to *Psiguria sp.* (Neck. ex Arn.) Cogn. (Cucurbitaceae), so for the analyses, only this species was taken into consideration. Pollen identification was done by comparing microscope photographs of pollen samples directly collected from *Gurania* sp.*, Psiguria* sp. and *Psychothria* sp. from the surrounding areas, which are naturally preferred host pollen food plants for *Heliconius*. Counts of 250 pollen tetrads were done per slide and the tetrads were classified as "intact" if the exine was intact and if their content was as homogeneous as in previously observed pollen from the anthers of the respective flower (Krenn *et al.* 2009) (Figure 2). Pollen grains were classified as "damaged" if they were deformed, had heterogeneous content, or if the pollen exines were broken and/or empty (Krenn *et al.* 2009) (Figure 2). Tetrad counts were done with Adobe Photohop CS3 (version 10.0.1) by overlapping a digital grid over each photograph and then marking each tetrad with a color dot: blue for intact and red for damaged; this technique assured zero error in the counts. A maximum of two individuals were sampled per site per day to avoid disturbing the butterflies. Usually if there are repeated disturbances at roost sites, the butterflies will abandon the site and relocate (per. obs.). Only individuals bearing pollen loads were used. Eighteen samples were collected at 3 roost sites over a 30-day period. In addition, 15 individuals that were observed bearing pollen loads while perched after dusk were marked with a permanent marker on the wing and observed the next day before leaving the roost for evidence of any pollen in their proboscis. Every individual used for pollen samples or marked was sexed.

## RESULTS AND DISCUSSION

All 33 *H. sara* individuals found at roosting sites bearing pollen loads were females. Three roosting sites were sampled on a daily basis and on average each site had 2-3 females and 4-5 males. All counts presented at least 50% of the pollen tetrads with damage (Figure 3-1). However, when comparing percentage of damaged pollen tetrads between the two collection periods, there are obvious differences. The first period (1630-1740 h) had a mean of 67.6% of damaged tetrads while the second period (2000-2200 h) had a statistically higher average percentage of damage 85.1% (t-test $P<0.01$). In addition, the 15 marked individuals that were observed bearing pollen loads after dusk had no pollen on their proboscis before leaving the roost next day. Normally after the pollen load has been digested, it is passively discarded (Krenn *et al.* 2009). It is clear, then, that only females are digesting pollen overnight. *Heliconius* butterflies start to actively forage during the morning, when *Psiguria* sp. inflorescences open and have maximum levels of pollen and nectar. This trend is supposed to reduce pollen availability on *Psiguria* sp. flowers, which don't produce nectar or pollen in the afternoon. However, there are no accurate data on how the pollen levels of *Psiguria* sp. flowers change through the day. *Heliconius* butterflies digest and discard pollen within several hours so it is unlikely that the analyzed pollen loads came from early morning collection events. Pollen amount was not recorded but the pollen loads observed and analyzed were comparable to the ones seen regularly in the mornings. This, together with the fact that about 50% of all the pollen analyzed had some level of damage by the time the butterflies start arriving to the roost sites, suggests that *Heliconius* females continue to find *Psiguria* sp. and efficiently forage until 1400-1500 h, and possibly just before starting to head over to roosting sites. It is also important to add that *Psiguria* sp. flower availability can be affected by a number of factors including rain levels (Murawski 1987), natural disturbances, and anthropogenic disturbances.

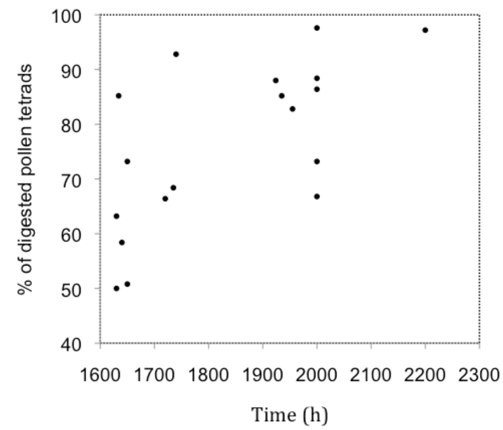


Fig. 1. Percentage of damaged *Psiguria. sp* pollen tetrads from samples collected from *Heliconius sara* individuals at roosting aggregations in La Selva field station, Costa Rica.

The different levels of pollen damage found in the samples collected at dusk probably reflect digestion efficiency and intra- and interspecific competition among *H. sara* females (Boggs *et al.* 1981, Cardoso 2001).

Diurnal observations have shown that females tend to carry larger pollen loads than males (Gilbert 1972, Boggs *et al.* 1981). This is linked to the female's physiological and ecological requirements, because pollen provides nutrients for egg production, increased longevity (Gilbert 1972, Dunlap-Pianka *et al.* 1977, Boggs and Gilbert 1979, Boggs *et al.*1981, Cardoso and Gilbert 2007), and for *de novo* synthesis of cyanide glycosides (Nahrstedt and Davis 1983). The results here support these studies by providing evidence that *H. sara* females forage more intensively than males and suggest that nocturnal digestion could be a strategy to maximize pollen feeding benefits. Additional observations in Pico Bonito, Honduras, revealed that *H. charithonia* females also arrive at roosting sites bearing pollen loads.

Despite the likelihood of nocturnal digestion as an advantageous adaptive strategy for *Heliconius* females, it is unclear whether this strategy is the result of abundant *Psiguria* sp. availability in the study area. *Heliconius erato*, another roosting species, has never been seen consistently (in periods of at least 30 days, in two different years) bearing pollen loads at roost sites found in Panama.

The findings presented here show a potential key strategy that *Heliconius* females may use to maximize pollen feeding. Further field research on *H. sara* and other roosting and non-roosting species is needed to determine if: (1) roosting environmental conditions offer specific advantages

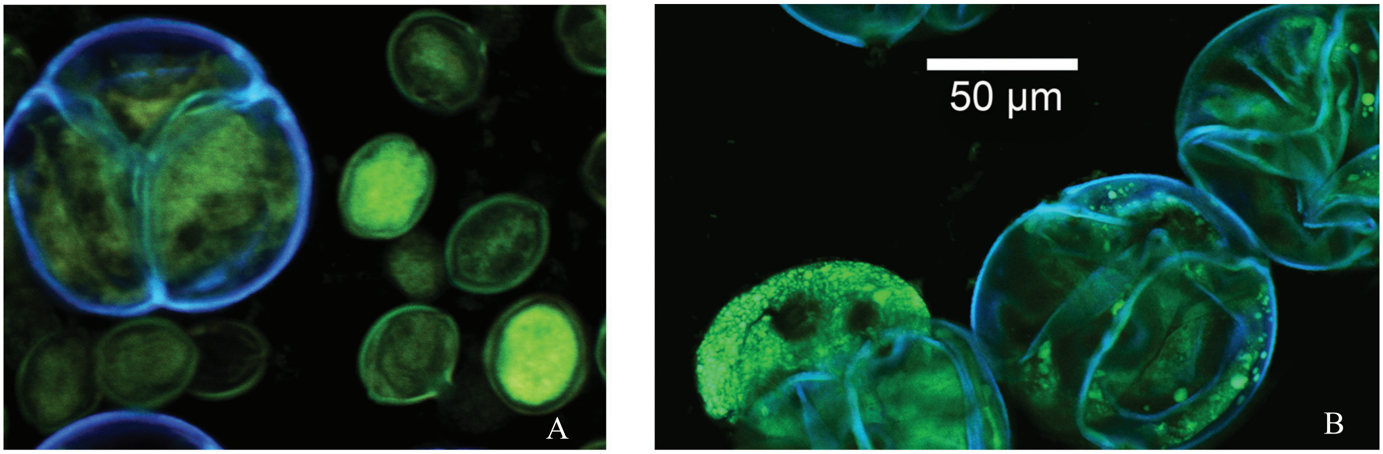


Fig. 2. A: Undigested *Psiguria* sp. pollen tetrad removed from a *Psiguria* sp. flower. B: Digested pollen tetrads removed from the proboscis of a *Heliconius sara* ♀.

for optimized pollen digestion, (2) nocturnal pollen feeding is spread among other roosting and non-roosting species, and (3) to reveal if there are correlations between nocturnal pollen feeding and local availability of pollen sources.

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