A Potential for Reproductive Diapause in Neotropical Butterflies

Kyri Forman [1, 2]

[1] Department of Biology, Utah Valley University, Orem, Utah, USA
[2] Alliance for a Sustainable Amazon (ASA), Las Piedras, Madre de Dios, Peru

Corresponding emails: forman.kyri@gmail.com & info@sustainableamazon.org

Abstract

Peru's Madre De Dios Region has some of the highest biodiversities of Lepidoptera species found globally. The rainforests of this southeastern part of Peru also have distinct wet and dry seasons, impacting many of the numerous organisms found there. For many species of Lepidoptera present in this region, necessary host plants may dry up or become otherwiseinaccessible. This would then lead to a period where conditions are unfavorable for some speciesfor some part of the year. In more temperate areas, other species of butterflies will undergo reproductive diapause, a strategy to wait out similar, unfavorable conditions caused by seasonal changes. This research sets out to find signs of reproductive diapause in neotropical butterflies during the first part of the dry season. The main subfamily studied was the Satyrinae (L:Nymphalidae) as many of these species are known to feed on grasses, which are often some of the first plants to go dormant during the dry season. Individuals were collected during the monthsof June and July and dissected to analyze for signs of reproductive diapause. Potential signs of reproductive diapause were seen in six species of Satyrinae as well as in *Eunica pusilla* (Nymphalidae: *Biblidinae*), signifying a possibility of reproductive diapause being used as a strategy in neotropical butterflies, a previously unknown phenomenon.

Introduction

The Amazon rainforest makes up the largest rainforest in the world and because of its geography, it contains many gradients in both temperature and moisture levels across the area. In southeastern Peru, there is a pronounced dry season from June to September, and during these months, a percentage of the vegetation will go dormant, die back, or shed their leaves. However, Peru and other parts of the western edge of the Amazon basin are also the most biologically diverse places for Lepidoptera species. This loss of vegetation may potentially affect various butterfly species as many depend solely on specific host plants, with some Lepidoptera only specializing in as little as one or two species of plants. When the availability of these hosts diminishes, some species of Lepidoptera may undergo various types of survival strategies similar to arthropods found in more temperate areas. Traits such as diapause or migration are commonplace for insects and other arthropods in temperate zones but have proven to be quite understudied in more tropical areas.

Diapause is a common trait found across many temperate species of insects and other arthropods. It is the arrested state of development used by many insects to help survive unfavorable conditions that occur annually in these zones. Diapause is species-specific and comes in many different forms, depending on the life stage it is present. Most forms of diapause such as those occurring in the egg, larvae, or pupal life stages cease growth and cause a failure to molt. The immature then can "wait it out" until environmental conditions improve allowing diapause to be terminated. Once diapause is terminated, growth and development are resumed, allowing the individual to reach adulthood and reproduce during a prime time of the year. In adult diapause, the development of the reproductive organs is ceased.
Reproductive organs stay small and undeveloped which causes a failure to produce eggs in females and sperm in males and lasts from the time the individual emerges as an adult until seasonal environmental cues terminate it. This allows more fats to be stored in the abdomen as there is less space used for the storage of eggs or other reproductive organs. Reproductive diapause also typically lengthens the lifespan of individuals as energy is not being converted to reproduction but to survival and/or migration.

Diapause is a ubiquitous trait across temperate insects and other arthropods (Denlinger *et al.*, 2011), and diapause in temperate species of butterflies is well studied. Diapause is used in butterflies and other insects in temperate zones to survive the harsh un-liveable conditions that are present for a significant part of the year. Such a trait is often induced by ambient environmental conditions that are measured by the insect and are used as cues to control the onset and the termination of diapause. The most well-understood environmental cue is photoperiod, the length of daylight a given area receives, as it is the most studied and possibly the most common environmental cue to trigger diapause. Other cues such as temperature, host plant quality, and humidity have been reported as being used as well.

While much is known about diapause and other seasonal traits in temperate species, there is little information on seasonality in neotropical butterflies (Jones and Rienks, 1985; Braby and Jones, 1995).
There have been some reports of diapause in many tropical Australian butterflies such as *Euploea core* and *Euploea sylvester* (Canzano *et al.,* 2003) and in other species of Satyrinae in tropical savanna (Braby, 1995). It has also been suggested that *Hypolimnas bolina* undergoes reproductive diapause during the dry winter months in Northern Australia (Kemp, 2001) and the termination of such diapause is caused by an increase in moisture levels at the end of the dry season. While there are some examples of reproductive diapause in tropical Australian butterflies, and other seasonal traits in African species, there are no reports of diapause in Neotropical butterflies.

The aim of this research is to search for any butterfly species -with a focus on the subfamily Satyrinae- located at the Finca Las Piedras Field Station that may show signs of reproductive diapause, and therefore obtain a sample of neotropical species that may have a potential for seasonal adaptations.

.

Methods

Specimen collection

Adult Satyrinae of various species were collected during the months of June and July with a hand net in various habitats. Since the leaf quality of host plants is more likely to

be affected during the dry season, specimens were collected during these months, from the beginning to almost the peak of the dry season in the Madre de Dios Region in Southeastern Peru. Habitats consisted of open, disturbed areas across the Finca Las Piedras Research Station; a man-made open forest consisting of mainly Cacao trees; along the trails of a selectively logged secondary forest; and along a dirt road surrounded by the same selectively logged secondary forest. Each adult collected was then given its own code and data such as date, location, habitat found, specimen condition at capture, subfamily, and species (if known) was recorded.

Unica pusila (subfamily: Biblidinae) was also used in this diapause study. Both males and females were collected bi-weekly across late June and the month of July. All *Unica pusilla* specimens were collected in open/disturbed areas across the Finca Las Piedras Research Station.

Diapause determination

To determine diapause, abdomens were removed from adult specimens for dissection. Each abdomen was then suspended in a labeled centrifuge tube with a solution of roughly 1-2% tribasic sodium phosphate dissolved in water. Tubes were placed in near-boiling water for 10 minutes to soften the abdomens for dissection. Abdomens were then removed from the tubes and dissected in water under a microscope. Diapause was determined in males by the presence or absence of a -currently unidentified- red organ found under the fourth segment from the end of the abdomen and in females by the presence or absence of eggs. If eggs were present, then the number of mature eggs was counted.

Results

A total of 186 Satyrinae individuals making up roughly 12-16 species were collected with a hand net in mixed habitats. 145 of these were later dissected for signs of diapause, and species with only one individual collected were not used in this study. Signs of diapause were observed in 6 species of Satyrinae and in *Eunica pusilla* (subfamily: Biblidinae). In most of the species in this study, only 1-16 individuals were collected, while in others (species 1 and *Eunica pusilla*) 60 and 40 individuals were dissected respectively.

Species 1

Diapause		No Diapause	
Male	3	Male	38
Female	4	Female	15
Total	7	Total	53

Species 2 - Pareuptychia

Diapause		No Diapause	
Male	8	Male	3
Female	4	Female	4
Total	11	Total	7

Species 3 - Cissia

Diapause		No Diapause	
Male	3	Male	7
Female	3	Female	3
Total	6	Total	10

Species 4

Diapause		No Diapause	
Male	2	Male	0
Female	1	Female	0
Total	3	Total	0

Species 5

Diapause		No Diapause	

Male	4	Male	0
Female	1	Female	4
Total	5	Total	4

Species 6 - Pierella

Diapause		No Diapause	
Male	6	Male	2
Female	0	Female	0
Total	6	Total	2

Species 7 - Eunica pusilla

Diapause		No Diapause	
Male	5	Male	0
Female	35	Female	0
Total	40	Total	0

Discussion

The results of this study suggest that several species of neotropical butterflies undergo reproductive diapause during the dry season. *Eunica pusilla* (subfamily: Biblidinae) shows the highest potential of being in reproductive diapause as (1) all 40 individuals (35 females, 5 males) dissected showed signs of diapause in that females had no eggs present, and males were missing the visible red colored testes found in other non-diapausing species. (2) Both males and females had more fat storage in the abdomen. (3) It should also be of note that *Eunica pusilla* seen here at Finca las Piedras was showing migratory behaviors such as directional flight and sudden appearance in mass numbers (Geoff Gallice,

currently unpublished work). Reproductive diapause has been reported in other migratory insects such as the monarch (*Danaus plexippus*, L. Nymphalidae) and (). (4) It was also observed that across the thousands of *E. pusilla* seen in the camp, no individuals were expressing reproductive behaviors such as ovipositing or mate-locating.

In the other species observed (subfamily: Satyrinae), only one had shown ubiquitous signs of diapause, but only three individuals were caught and dissected. In the other species, mixed results were seen (species 1 (7/60); species 2 (11/18); Species 3 (6/16); Species 5 (5/9); Species 6 (6/8)). This could be because what is labeled as a single "species" might be a combination of multiple cryptic-looking species instead of just one. If this is the case then it is possible one species in a group is in reproductive diapause while other similar-looking species are not. This could be solved by completing a DNA analysis, to confirm individuals to a species or subspecies level.

Another theory presented here is that many species in the tropics may have phenotypic plasticity allowing the "choice" of entering reproductive diapause to be on a more individual level instead of being on a population or species level. This would lead to only a percentage of individuals being in reproductive diapause at a given time instead of an entire population. It is possible that such a phenomenon would be more common in tropical areas where seasonal conditions can be less extreme than in more temperate zones. Having only a few individuals breeding during a time like the dry season could be beneficial when conditions such as host plant availability are diminished, but not completely gone.

There is very little if no information known about the presence of reproductive diapause in neotropical butterflies. Therefore, even a base knowledge of the presence of diapause will be greatly beneficial to know how species may need to adapt to tropical climates. It will also be critical in knowing which species may be more likely to be adaptable to our changing climate. Those that have the ability to undergo diapause may be more likely to survive harsher conditions brought by climate change or be negatively impacted by the loss of reliable environmental cues.

There is much more that needs to be done to be able to make any strong conclusions. More specimens need to be dissected from other places and at other times of the year to determine differences between seasons or regions. There is also a very noticeable lack of information known about these species such as host plants, distributions, other seasonal patterns, and more. Without this information, it is difficult if not impossible for us to make any strong conclusions as to which species may be in reproductive diapause and the reasoning behind the need to do so.

Literature Cited

Denlinger DL, Yocum GD, Rinehart JP. Hormonal control of diapause. In: Gilbert LI, editor. *Insect Endocrinology.* U.K: Academic Press; 2011. pp. 430–463.

Jones, R. E., Rienks, J., and Wilson, L. (1985) Seasonally and environmentally induced polyphenism In *Eurema laeta lineata* (Lepidoptera: Pieridae). J. Aust. Ent. Soc. 24, 161–167

Braby, M. F., & Jones, R. E. (1995). Reproductive Patterns and Resource Allocation in Tropical Butterflies: Influence of Adult Diet and Seasonal Phenotype on Fecundity, Longevity and

Egg Size. *Oikos*, *72*(2), 189–204. https://doi.org/10.2307/3546221

Canzano, A. A., Jones, R. E., and Seymour, J. E. (2003). Diapause termination in two species of tropical butterfly, Euploea core (Cramer) and Euploea sylvester (Fabricius) (Lepidoptera: Nymphalidae). *Australian Journal of Entomology*, 42, 352–356.

Braby, M. F. 1995. Reproductive seasonality in tropical satyrine butterflies: strategies for the dry season. Ecol. Entomol. 20: 5–17.

Kemp, D. J. (2001). Reproductive seasonality in the tropical butterfly Hypolimnas bolina (Lepidoptera: Nymphalidae) in northern Australia. *Journal of Tropical Ecology*, 17:483–494.

Jones, R. (1987). Reproductive strategies for the seasonal tropics. *International Journal of Tropical Insect Science*, *8*(4-5-6), 515-521. doi:10.1017/S1742758400022566

Denlinger, D. L. (1986) Dormancy in tropical insects. A. Rev. Ent. 31, 239–264.

Jones, R. E. and Rienks, J. (1987) Reproductive seasonality in the tropical genus *Eurema*. Biotropica 19, 7–16.

Wolda, H. (1978a) Seasonal fluctuations in rainfall, food, and abundance of tropical insects. J. Anim. Ecol. 47, 369–381.

Hiroyoshi, S., & Reddy, G. (2018). Field and Laboratory Studies on the Ecology, Reproduction, and Adult Diapause of the Asian Comma Butterfly, *Polygonia c-aureum* L. (Lepidoptera: Nymphalidae). *Insects*, *9*(4), 169. https://doi.org/10.3390/insects9040169