Protocol for monitoring *M. leda* phenotypes

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Introduction

The optimal colorations of animals vary in space and time, depending on e.g. habitat background coloration, predator community composition, the animal's activity pattern, and the colorations of alternative prey animals. Short-lived insects can use seasonal polyphenism to express different colorations in different seasons. In addition, local selection can lead to regional differences in average colour patterns. In some cases, species have evolved polymorphism so that local models are mimicked, or in the case of species that rely on crypsis, polymorphism that avoids search-image formation in predators.

We plan to study one of the most widespread butterfly species, the common evening brown *(Melanitis leda)* which occurs throughout the tropics and sub-tropics except in the Americas, from forest to grassland habitats, and often at high densities. It shows seasonal polyphenism, and the dry season phenotype is polymorphic in wing pattern and colour traits. We will study various aspects of local adaptation in common garden experiments as well as the genetic basis of these traits. In addition, we would like to obtain insight into polyphenism and polymorphism throughout the range of the species. We are also interested in patterns of wing damage because that contain information on interactions with predators. Therefore, we are looking for collaborators who can photograph *M. leda* monthly for one year, and if possible provide a few specimens for genetic analyses, across the entire range of this species.

Wet season form Dry season form

Fig. 1: Wet and dry season forms of *M. leda*. Wet season forms have large eyespots and the background is evenly covered with irrorations (stripy pattern), with sometimes faint darker bands. Dry season forms have much smaller and less contrasting eyespots, the background colorations are variable, the forewing tips are falcate, and the tails are longer.

In addition to the focal species, we are also interested in other species, such as other Melanitini and in Mycalesini. Once you have put the traps and went there to photograph the target species, why not photograph or score all the butterflies? These data will be useful to study wing damage patterns, phenotypic plasticity in body size, and butterfly community structure.

Methods

Site selection

The study site does not need to be natural, but can include gardens and plantations. Fruit-baited traps are usually the most efficient and allow easy sampling of most fruit-feeding butterfly species (Figure 2). Butterfly traps consist of a mesh cylinder with a closed top and an open bottom with a bait platform below.

Fig. 2: Butterfly trap made of wooden embroidery hoops, hard netting, plastic plates and rope. See the documents about traps for more information.

Traps are typically hung along two transects of 10 traps each with a distance between traps of at least 30 meters. Different arrangements are possible in this project after consultations with the PI or co-PI. Traps are hung from branches of trees or large shrubs using a short rope. The bait platform should be about 40 cm from the ground. Traps tend to be more effective when hung near gaps in the canopy, but it is better to avoid full sun. Avoid problems with ants by hanging traps from trees and branches that are not frequented by ants, and avoiding hanging traps from thick branches and vines that ants use to travel between trees. If ants do enter the trap and predation on butterflies was detected (remains of butterfly wings in the trap), the trap position needs to be changed to a different tree nearby. Also make sure that the vegetation does not touch the bait platform or trap (remove plants touching the trap if necessary), even in case of wind. In the unlikely event that bats start raiding the trap, it needs to be moved at least 20 meters. The trap is baited with two serving spoons of bait put into a small bowl placed on a bait platform. Make sure not to touch the traps fabric with bait or spill bait near the trap, otherwise butterflies will feed outside the trap instead of entering it. Each month, the habitat at the immediate vicinity of each trap should be photographed (a photograph of the trap taken from a distance of about 5 m). You can change the photo name to the trap name. Each trap needs to be numbered and the coordinates noted. You can use a smartphone application (e.g. Compass) to get the coordinates and often location is also stored with photographs taken. Store the numbered traps of a transect in order in a bag so that they are easy to install in order.

Timing

We are aiming for 50 specimens of *M. leda* per month for one year for a given location, but fewer are still of interest. Typically at a given site we perform trapping once a month for three days (so 4 days fieldwork), but it also possible to e.g. perform (almost) daily trapping with fewer traps such

as in a garden. In case of heavy rain or strong wind, you need to postpone trapping or select additional trapping days. The traps are kept baited overnight as they tend to be active during the evening and morning. It is more effective to start checking the traps later in the day (e.g. at 11 AM). In case *M. leda* is at low density but not (nearly) absent (5-10 per day at a transect), adding additional trapping days is recommended to collect a sufficient number. The populations of *M. leda* tend to fluctuate, so they may often be common, but can at time be rare.

Preparing bait

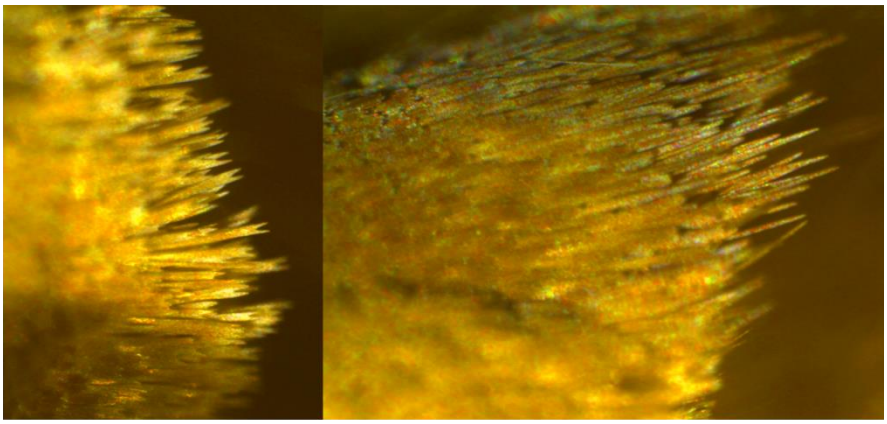
Butterfly traps are baited with fermented mashed-banana. Overripe bananas are cut into pieces and mashed in a bucket using a stick. The bait needs to be prepared at least 2 days in advance and kept at a warm temperature to ensure proper fermentation. The lid of the bait bucket can be left slightly open to allow gasses to escape.

Recording data

Even though the project focuses on *M. leda* we suggest to record all butterflies that are caught in the trap. This is relatively little extra work but provides rich data about the butterfly community including also information on seasonal polyphenism in other species and wing damage patterns. Each specimen should be photographed with sex information and include standards into the image (size reference and grey, black, and white card pieces). For that purpose, we developed a grey card with a piece of white card and a piece of lack card on each site, one side for males, the other for females (Fig 3). You then flip the card depending on the sex of the specimen.

Fig. 3. Grey card with white and black standard as well as sex information. The other side of the card looks the same but has a male symbol. If the specimen has wing damage, it should be photographed from both sides. The card should be cleaned with soapy water after each day of usage. We avoid collecting data on the parts covered by the thumb.

M. leda can be sexed reliably by inspecting the tip of the abdomen (Fig. 4). Males have filiform scales at the tip of the abdomen which can be perceived as tiny 'hairs', while female have regular forked scales and thus lack such 'hairs'. A magnifying glass may be necessary, but it will be easy after a while. In Mycalesines (e.g. *Bicyclus* and *Mycaleses*), males have a silvery area where the fore and hindwing overlap, and males have androconial hairs (scales) or patches. To identify recaptures easily, released butterflies should be marked with a permanent marker on the left ventral forewing.



Female Male

Fig. 4. Close up of the tip of the abdomen of a *M. leda* female and male.

Enjoy the trapping!

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