

Stable isotopes reveal the ecology of tropical butterfly larvae

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Despite many ecological studies, our understanding of the larval ecology of many tropical butterfly species is very limited. In contrast to adults, caterpillars are mainly active at night and are notoriously hard to spot in the wild. This seriously hampers investigations of the evolution of feeding ecology and potential co-evolutionary interactions between host plants and butterflies. One group of butterfly species that is particularly interesting in the context of plant-herbivore co-evolution is the subtribe Mycalesina (Nymphalidae). These tropical butterflies feed mainly on grasses as larvae and have radiated dramatically in Sub-Saharan Africa, Madagascar and Asia with over 300 extant species.

The commonest form of photosynthesis is called C3, but tropical grasses often have a variant called C4, and the evolutionary history of mycalesine butterflies is expected to be closely tied to the rapid replacement of C3 vegetation by C4 grasslands in the Late Miocene-Pliocene. In contrast to C3 host plants, these C4 grasses tend to have a higher physical toughness and lower nutritional values. Therefore, insect herbivores, such as mycalesine larvae, are expected to avoid C4 grasses when C3 host plants are available. In addition, one would predict that novel feeding adaptations, for instance in jaw morphology or digestive physiology, may have evolved in mycalesines in response to the ecological dominance of C4 grasses in open savannah habitats.

Stable isotope analyses represent an unexploited opportunity to increase our understanding of the larval ecology of mycalesine butterflies. The environmental conditions to which a caterpillar is exposed are imprinted into



Ecologists at work. Photo provided by authors.

the exoskeleton of the adult during metamorphosis. By measuring the ratios of several stable isotopes we can recover the information that is 'recorded' in leg material (=exoskeleton) of adult butterflies, which are relatively easy to capture in the wild. Here, we show that stable isotopes of carbon provide information about the type of host plant, C3 or C4, which was used by the larvae. In addition, stable isotopes of oxygen reveal how much water vapour was present in the atmosphere during larval development.

The isotopic composition of leg tissue in mycalesine butterflies shows that species that inhabit open C4 grass-dominated environments are quite opportunistic in their larval host plant choice. Nevertheless, we observe that during the dry season, larvae are more likely to use high quality C3 grasses and are exposed to lower levels of water vapour in the atmosphere. Finally, our data reveal that the ability to process C4 grasses is clustered within related groups of mycalesine species, suggesting that C4 grass processing adaptations may have evolved in this subtribe.