

Motion analysis of butterflies: A method to investigate the evolutionary origin of the unique pollen feeding behaviour of *Heliconius* butterflies (Nymphalidae)

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Pollen feeding behaviour is unique to butterflies of the neotropical genera *Heliconius* and *Laparus* (Heliconiinae, Nymphalidae) and may be key for understanding life-history evolution [1]. Like many other butterflies they feed on floral nectar but they actively collect pollen on their proboscises and use it as an additional food source. Amino acids are extracted from the pollen grains by way of an extra oral digestion involving saliva [2] [3]. The mechanisms and the evolutionary origin of this special behaviour are unknown.

Here, we address the idea that pollen feeding behaviour may have originated from proboscis cleaning behaviour by using motion analysis. We compared mouthpart movements of 9 butterfly species (6 individuals each) of different relatedness [4]: 5 pollen feeders of genus *Heliconius* (*H. cydno*, *H. hecale*, *H. melpomene*, *H. pachinus* and *H. sara*) as well as 2 closely related species, pollen feeding *Laparus doris* and non pollen feeding *Eueides isabella*; *Dryas julia*, a related non pollen feeding Heliconiinae; and non pollen feeding species *Anartia fatima* as nymphalid out-group. All species were collected in the field near the “Tropical Station La Gamba” in Costa Rica and set free after use in experiments. To initiate pollen extraction behaviour or proboscis cleaning behaviour pollen or small glass beads (ca. 106µm) were placed on the proboscises of all butterflies. The behaviour of butterflies was recorded on video tape with a JVC GZ-MG37E hard disc camcorder for 20 min each. Three distinct proboscis movements, 5 different degrees of proboscis extensions, movements of the entire butterfly and the release of saliva were coded from video tape by using “The Observer XT” [5] [6]. All movements and the release of saliva were coded as state events, with a start point and an end, for comparing the quantity and the duration between species.

Preliminary results show that similar patterns of movements are employed by pollen feeding and non pollen feeding

butterflies suggesting that the evolutionary origin of pollen feeding behaviour is likely a modification of proboscis cleaning behaviour. Furthermore there are also indications that the key innovations of pollen feeding behaviour are the frequency of the proboscis movements and the repeated release of saliva during pollen extraction. Motion analyses using “The Observer” software make it possible to investigate the pollen feeding behaviour of *Heliconius* butterflies on a fine scale and therefore new insights in the evolution of this unique behaviour can be achieved.

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