Motion analysis of butterflies: Investigation of the evolutionary origin of pollen feeding behaviour of Heliconius butterflies (Nymphalidae).

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Summary
Pollen feeding behaviour of Heliconius and Laparus butterflies (Nymphalidae) represents a key innovation for their advanced life-history. After collecting pollen on the outside of their proboscis, a particular pollen processing behaviour extracts amino acids from the pollen grains. In a comparative approach the proboscis movements are described in various Heliconius species.

We studied the proboscis movements of pollen processing using experiments and video footage in various species of pollen feeding and non pollen feeding butterflies. Triggered by the presence of pollen on the proboscis, all examined species performed a set of similar proboscis movements. Differences occurred in the frequency between pollen feeders and non-pollen feeders.

Methods
We compared mouthpart movements of 9 butterfly species (6 individuals each) of different relatedness [4]: 5 species of pollen feeders of the genus Heliconius (H. cydno, H. hacaule, H. melpomene, H. pachinus and H. sara), the pollen feeding Laparus doros and the related non pollen feeding Euodes isabella and Dryas julia, the latter subfamily Heliconiinae, and non pollen feeding species Anartia fatima as nymphalid out-group. The experiments with H. cydno and H. melpomene were conducted in the green house of the University of Texas, Austin, Brackenridge Field Laboratory. 11 Heliconius butterflies voluntarily collected pollen from flowers as a natural act.

Results & Discussion

The subsequent pollen processing behaviour was recorded. All other species were collected in the field near the “Tropical Research Station La Gamba” in Costa Rica and set free after the experiments. To initiate pollen extraction behaviour or proboscis cleaning behaviour commercially available pollen or small glass beads (ca. 106 µm) were placed on the proboscides of all butterflies. The behaviour of all butterflies was taped on video tape using a JVC GZ-MG37E hard disc camcorder recording for 20 min each. Three distinct proboscis movements (coiling, antiparallel and nothing), 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.

In addition a fine scale analysis of pollen extraction behaviour of 11 video tapes 2 min each was made to get a steady set of movements as a definition of pollen extraction behaviour. Detailed analyses of the coiling movement was achieved by distinguishing between the in and out coiling as well as up and down movements of the entire proboscis. All of these behaviours were coded as state events.

Proobsis movements during pollen processing
Three categories of motions can be distinguished. The different subcategories or intensities are coded with different extension categories of the proboscis.

a. Coiling. The proboscis spiral is partly coiled from the tip to its peripheral parts and subsequently partly uncoiled. b. Uncolling (as subset of a). The proboscis is uncoiled into an extended position. c. Antiparallel movements. The proboscis halves are moved against each other, the partly coiled proboscis is slightly moved sideways. d. Up-and-down. The entire proboscis is raised and lowered at its joint to the head.

Laparus doris collecting pollen

Introduction
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 proboscides 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 evolutionary origin of this special behaviour is unknown.

Here, we address the hypothesis that pollen feeding behaviour may have originated from proboscis cleaning behaviour. Comparative motion analysis using "The Observer XT" provides detailed information about duration, frequency and the sequence of different events. The aim is to compare the movements of these two behaviours between butterfly species.

Literature