Hypothesis a nectar reward. materials and methods

Literature

HypothesisOur present project aims to study the individual role colour and shape of the flower play in food finding behaviour of a lepidopteran species. We hypothesize that the combination of both colour and shape would be the most effective way for a butterfly to locate its food, but one of the cues is learned readily than the other, it may serve as a primary cue in food finding.

One cue may dominate over the other and the differential role each cue plays in food finding would be elucidated from this study. Objective· To study the relative importance of colour and shape as cues used by butterflies for detection of flowers while foraging.· To examine whether Papilio polytes can learn to associate a compound stimulus, i. e. colour and shape together with a nectar reward. Materials and MethodsCollection and rearing of individualsEggs and larvae of a particular butterfly species would be collected and reared in laboratory under semi natural conditions. The hatched larvae will be fed on fresh leaves of host plants. Larvae would be allowed to pupate in greenhouse cage in temperature corresponding to field conditions.

The day pupa ecloses will be termed as post emergence day 1. For identification purposes, butterflies were marked with a unique number, on its forewing using a fine tip marker, at least 24 hours after eclosion. Experimental setupExperiments would be performed in a mesh cage containing the flower models. The project will be consists of behavioural experiments with the following experimental set-up: For determining colour preferenceWe selected yellow colour for its strong innate preference over green colour in earlier studies. Tests will be carried out 24hrs after the butterfly eclosed. The testing will take place in two different mesh cages

containing 16 radial shaped flower models (8 yellow and 8 green) and 16 bilateral shaped flower models (8 yellow and 8 green) respectively. Observations will begin immediately after the butterflies were placed in the cage individually, will last for 30 mins. Butterfly’s innate preference will be recorded as the first colour that it flies to and lands on in the array and not falls or walks towards.

Observations will include the first colour it landed on, time to locate the first flower and number of visits the butterfly makes to each colour. A butterfly that will not make a choice within 30 mins will be tested again in the next few hours. The flower models will be wiped with a brush after each trial. The effect of relative position of flower models on the feeding behaviour of the butterfly will be nullified by randomizing the position. The subjects will be starved for a fixed duration before the experiment. Each butterfly will be tested twice for the confirmation of the colour choice it makes, on radial flower shape as well as on the bilateral flower shape. For determining shape preferenceWe selected radial flower shape and bilateral flower shape. Tests will be carried out 24hrs after the butterfly eclosed.

The testing will take place in two different mesh cages containing 16 yellow colour models (8 radial and 8 bilateral) and 16 green colour models (8 radial and 8 bilateral) respectively. Observations will begin immediately after the butterflies are placed in the cage individually, will last for 30 mins. Butterfly’s innate preference will be recorded as the first colour that it flies and lands on in the array and not falls or walks towards. Observations will include the first colour it landed on, time to locate the first flower and number of visits the

butterfly makes to each colour. A butterfly that did not make a choice for 30 mins will be tested again in the next few hours. The flower models will be wiped with a brush after each trial. The effect of relative position of flower models on the feeding behaviour of the butterfly will be nullified by randomizing the position.

The subjects will be starved for a fixed duration before the experiment. Each butterfly will be tested twice for the confirmation of the shape choice it makes, on yellow flower colour as well as green flower colour. TrainingPost preference test we will randomly assign the butterflies to a particular training combination. These four different training groups of butterflies will be released in different mesh cages the same day of preference test. The training will be carried out in the mesh cages containing a host plant for egg laying and 16 flower models of different combinations (4 yellow bilateral, 4 yellow radial, 4 green bilateral and 4 green radial) with maximum of 8 butterflies per cage, both males and females.

Only the assigned training combination will have nectar inside the flower models and will be refilled regularly, others will be left empty. We will start training with feeding them for 5 seconds by gently unrolling its proboscis, on the assigned training combination thrice a day for 8 consecutive days, to reinforce the training shape and colour. The butterflies will be free to visit any flower model during this period and will be able to train themselves to the assigned combination. The location and arrangement of the flower models will be randomised every day. TestingButterflies will be tested after 8 consecutive days of training followed by a fixed period of starvation.

Before testing each butterfly will be fed on the assigned training combination for 5 seconds to encourage feeding behaviour, a standard practice in butterfly training. Each butterfly will be then released into a cage containing an array of 4 yellow bilateral, 4 yellow radial, and 4 green bilateral and 4 green radial flower models without nectar. (With a feeder with black colour star shaped flower model as a control for the feeder??) and observations will begin immediately. Observations will include the colour and shape of the 1st flower model visited, the time to locate the flower model as well as the number of visits and the amount of time it spends probing on all the flower combinations.

The test will last for 30mins. Prior to the experiments, various behavioural responses will be defined. A positive response to any combination will be defined by a visit in which the butterfly hovers around the flower and extends its proboscis towards it. The effect of relative position of flower models on the feeding behaviour of the butterfly will be nullified by randomizing the position. Analysis of the data1.

Reflectance spectra of chosen colours2. Colour/ Shape preference tests – plots of Maximum no. of visits or 1st flower model located? 3. Tests- plots of the amount of time spent on all flower models by each training group of butterflies to find out if the reward was associated with colour or shape primarily.