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Hind Suhail Abdulhay
Department of Biology, College of Science, University of Baghdad, Iraq

Correspondence
Hind Suhail Abdulhay
Department of Biology, College of Science, University of Baghdad, Iraq

Defensive behavior in lemon butterfly *Papilio demoleus* L. (Lepidoptera: Papilionidae) against predation

Hind Suhail Abdulhay

Abstract
The present study investigated the effect of camouflage in larvae of lime swallowtail, *Papilio demoleus* L. as a prey on the predator behavior. Three experimental groups were done to find out whether young chicks, *Gallus gallus domesticus*, as predators can recognize and attack the initial larvae of *P. demoleus* L. that resemble bird droppings from the larvae frass, or the latest greenish fifth instar onwards from different backgrounds colors. Experiments were first conducted by introducing the young larvae either alone or mixed with frass or the frass only to the chicks. All the chicks have failed to identify the larvae from the frass. Second experiment on the fifth instar onwards larvae were conducted by putting them on different backgrounds colors, a closely matched or contrasted with the coloration of the prey. Chicks showed greater attack for prey on contrasting backgrounds, and did not recognized or attacked the prey on a matching background. Third experiment was tested the ability of the chicks to recognize and attack the two dimorphic colors of the pupae. The experiment provides evidence that prey on contrast background may produce better recognition and attack for the predators.

Keywords: *Papilio demoleus*, camouflage, *Gallus gallus domesticus*, predation

1. Introduction
Camouflage in butterflies is an adaptation that has a beneficial effect to avoid potential predators. The simplest form is copying the color of the background or matching their host plant like some caterpillars which can blend into their surroundings extraordinarily well or looks as an inedible object, like bird droppings. Some have dark and light stripes or having various patterns on the wings which render recognition of organisms difficult while resting. Protective coloration (crypsis) is noticed in the larvae of hairstreaks and skippers which resemble their host plants in color and pattern.
Papilio demoleus L. (Lepidoptera: Papilionidae) commonly known as the lime or citrus swallowtail, is a major pest of citrus throughout most of its world range, causing significant economic losses [1] and also because it shows rapid population growth under favorable circumstances [2]. In recent times has expanded its range into new areas following the introduction and cultivation of citrus [3].
Researches indicated that butterflies are potentially most vulnerable in the pupa stage and in order to escape from predators they have developed the ability to camouflage themselves by resembling their surroundings. This is also an example of protective coloration. Previous reports typically focused on the predator- prey interactions [4, 5, 6], but a little concern about the responses of predators to the defensive behavioral of the prey. The present experiment investigated the importance of the defensive strategies to avoid predation in the butterfly life cycle by studying the ability of predator recognition the prey and prey contrast against the background on predator attacking behavior.

2. Materials and Methods
2.1 Predator
As predators in the experiment, domestic chicks (*Gallus gallus domesticus*) from house rearing were used in the experiment. The chicks were housed in a cage with a 100×55× 20 cm with wooden sides and a steel-net floor covered with sawdust. The roof made partly of wood and partly of chicken wire. The floor of the cage was covered with sawdust, and a 60 W carbon light bulb provided heat [7]. The chicks used in the experiment were 2-3 week old of either sex and were food-deprived for 1-2 hours before exposure to prey and after the experiment they were returned to the cage.

2.2 Prey

The early larval instars and eggs of *P. demoleus* L. were collected from lemon and citrus field by the end of summer and first half of fall 2013 in Baghdad. The eggs were kept in small Petri dishes inside suitable plastic container covered with nylon mosquito net. Daily examinations were made during the entire life cycle, but in certain cases reading were done twice a day for egg hatching at higher temperature and every day at lower temperatures for pupation and adult emergence. A camel hair brush was used to handle the egg and small larvae. The adult were kept in a plastic cage (1m ×1m × 1m) covered from the sides and the top with nylon mosquito net. Adult offered sugar solution as supplementary food either in open glass container where colored cotton was placed, or in artificial flowers, in both cases adult feed vigorously. Fresh young twigs of sour orange *Citrus aurantium* L. was supplied everyday or as necessary. For early larval instars only growing point were offered to prevent wilting and to minimize handling and disturbance of larvae, the twig were inserted in vials of suitable size full of water and plugged with cotton. The containers were cleaned regularly from remains of food and the frass which were collected and preserved. Insect were reared in the laboratory at $27 \pm 2\ ^{0}C$ temperature and 70±5% R. H. and 16 L:8 D photoperiod [8]. All experiments followed the same physical conditions.

2.3 Experimental procedure

2.3.1 Predation on larvae resembling a bird dropping

The camouflage in the first, second, third and fourth larval instars means that the caterpillar looks like inedible bird droppings so, the ability of the predator to recognized and attack the prey from these instars larvae were investigated by two experiments. First experiment consists of three trials. First trial, 2 g larvae frass were placed in Petri dish with a diameter of 5 cm containing filter paper in the bottom and then introduced to one chick placed in a carton box (50 cm × 50 cm) the predator was exposed to Petri dish containing only 2 g frass only. Five larvae were mixed with 2g larvae frass and placed in Petri dish with a diameter of 5 cm containing filter paper in the bottom and then introduced to one chick placed in a carton box (50 cm × 50 cm). The chick was observed for 30 minutes and the number of eaten larvae was counted and recorded. Chicks that had not attacked the preys during the time were presented to second trials with another five preys placed in Petri dish only without any frass and observed for the same time. Third the predator was exposed to Petri dish containing 2 g frass only.

Second experiment was conducted by introducing the preys with the three different types at the same time to the chick by putting it on a circular dish (20 cm × 1.5 cm height) divided to three parts. Also, was noted for the same time. All experimental procedures were carried out under the same environmental conditions. If the chick recognize and eat the preys then it will be replaced with another starving chick. This was repeated for each of the three treatments, using different chicks each time. After each trial the preys were examined: if the cuticle was ruptured the insect was considered dead. Five replicates were used for each trial.

2.3.2 Predation on larvae fifth instar onwards

The camouflage in this life stage is a shade of green that matches their host plant. So to influence the conspicuousness of the prey in the experiment, we used three different colored paper backgrounds in the bottom of each Petri dish. One was ordinary white filter paper; the others were green and black. The green papers were chosen to match the color of the *P. demoleus* larvae as closely as possible and the experiments were carried out in three trials:

First, five larvae from the fifth onwards instars larvae were placed in each Petri dish with a diameter of 5 cm containing filter paper in the bottom of the dishes as a white background, and then introduced to one chick placed in a carton box (50 cm × 50 cm). The chick was observed for 30 minutes and the number of eaten larvae from each Petri dish was counted and recorded. Chick that had not attacked the prey during the time was presented again to other preys with another background. Each trial was replicated five times.

Second part of the experiment each background color was further joined together into a circular dish (20 cm × 1.5 cm height) divided to three parts each containing three preys on three different backgrounds and introducing it at the same time to the chick. All experimental procedures were carried out under the same time and environmental conditions.

2.3.3 Predation on pupae

The experiment was conducted by introduced either green or dried small twigs of citrus to a prepupae of *P. demoleus* placed in cylindrical glass (10 cm height × 7 cm diameter) in order to pupate and noticed the dimorphic color of the pupae. From the experiments we recognize two different colors to the pupae the green on the green young twigs and the pink-brown on the dried twigs.

The two dimorphic colors of the pupae with the young twigs were put in the soil and exposure to the chick and observed the predator behavioral. This was repeated five times, using different chicks. If the experimental bird attacked the larvae, that trial was terminated as soon as the bird had ceased handling the insect. After each attack, the prey with ruptured cuticle was considered dead.

3. Results and Discussion

3.1 Predation on larvae resembling a bird dropping

It has also been shown experimentally that avian predators cannot discriminate between larvae and frass. The chicks behaved similarly towards the three presentation types. Chicks in all treatments generally did not recognize the larvae resembling a bird dropping and therefore did not attack them as shown in figure 1.

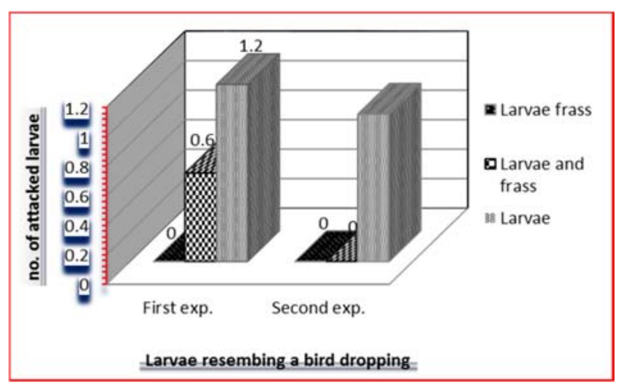

Fig 1: showed the 1st and 2nd experiment on larvae resembling a bird dropping

3.2 Predation on larvae fifth instar onwards

The experiment indicates the substantial effect of contrast background in chick recognition. The predator attacked the preys placed in white and black background in all replicates, while the prey in a similar background (green) generally did not attack as shown in figure 2.

It is important to say that some chicks even they attack the prey; they did not eat it but only probe the prey with their beaks then leave it. This may be due to the osmeterium gland just behind the head which found in larvae of the Papilionidae. When the larva is disturbed, osmeterium is everted, emitting a strong odor which repels the predators. It is also aposematically colored to deter the natural enemies. Also, the large conspicuous eyespots have evolved to defeat and discourage the predator attack because they mimicked eyes of the predators' own predators [9].

We used prey presented on different backgrounds, to produce a variation in conspicuousness. A contrasting background may increase the ability to see the prey and attack it. The time a chick has available to decide whether to attack a prey was also important. Young chicks make more mistakes in a choice situation between differently colored [10]. There is experimental evidence suggesting that birds associated unpalatability faster when the prey contrasts against the background than when the prey matches in coloration [11].

Chicks in all treatments generally attacked the *P. demoleus* larvae placed in black background before they attacked the prey on the white background. The background color affected attack latencies of the prey. Whereas contrasting larvae, on black background, were attacked faster by chicks, there was a significant effect of background color on prey (Figure 2).Thus, birds were more affected by background color, and they hesitated longer before attacking the prey when the coloration was matching the background than when the background was different. Preys were attacked significantly less often on matching background than on contrasting background. All chicks attacked larvae without discriminating between the two backgrounds. A more probable explanation for the effects of prey contrast against the background in the present experiment would be that it affected the birds' ability to recognize the prey as such and the black and white background paper produced faster and more attacks by the chicks [12]. Suggested that particular hues or intensities of color may be more effective than others, and accordingly birds seem to show differences in at least unconditioned aversions to certain colors and patterns often used in warning coloration [13].

3.3 Predation on pupae

This study provides further evidence of benefits of a matching coloration in prey. The chicks in all treatments generally did not recognize the pupae from the twigs either the green or the dried. Pupae are mostly cryptically colored or placed in inconspicuous positions on low growing plants or in cocoons. Many pupae are remarkably well protected by their color and forms. For example, the pupae of the orange tips resemble seed-pods and those of the Vanessidi often resemble a withered leaf both in shape and color.

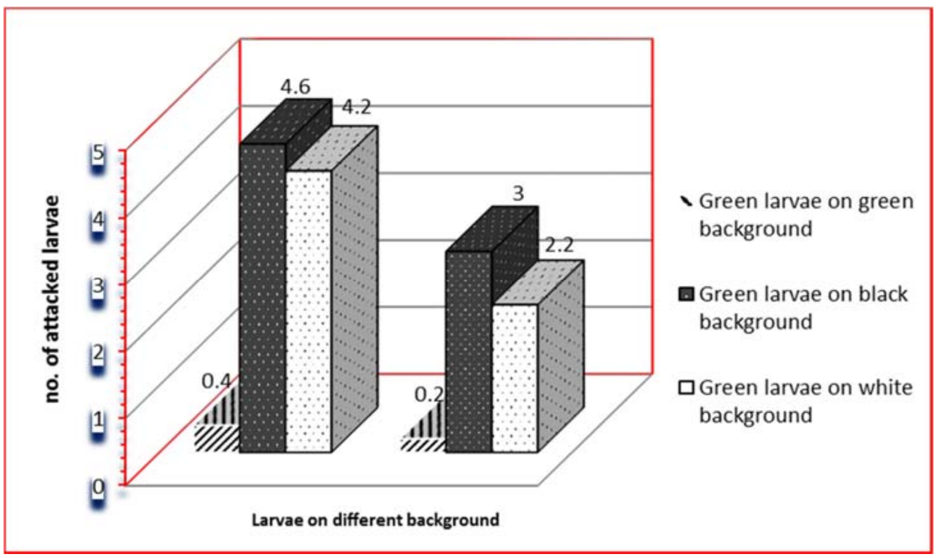

Fig 2: showed the 1st and 2nd experiment on fifth instar larvae onwards

4. Conclusion

Results of the study revealed the importance of camouflage in *P. demoleus* to avoid predation. The chicks did not recognize the initial larvae that resemble bird droppings when they were mixed with larvae frass. The ability of predator to distinguish the prey on different backgrounds colors was increased with contrast background. Also, the two dimorphic colors protected the pupae from predation as the chicks could not recognize the pupae from the green or the dried twigs**.**

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