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RESEARCH ARTICLE

THE BIOLOGICAL DIFFERENCES BETWEEN PUPA OF TWO SPECIES OF LADYBIRD *Hippodamia variegata* AND *Exochomus pubescens* (COL.: COCCINELLIDAE)

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ABSTRACT

A study on differences between two species of ladybird, *H. variegata* Goeze and *E. pubescens* Kuster determined that there were differences in terms of size, color of pupa shell, shape of pupa shell, pattern of adult emergence and length of adult from emergence out of the shell. In this research, pupa length of *E. pubescens* was 4.3 – 4.8 mm and pupa length of *H. variegata* was 3.6 – 4.2 mm. Shell color of *E. pubescens* pupa was pale brown or gray, but shell color *H. variegata* pupa was orange with black lines on it. Length of body in adult *H. variegata* after discarding the pupa shell was 3.7 – 4.2 mm and length of body in adult of *E. pubescens* after discarding the pupa shell was 3.7 – 4.1 mm. There were differences in appearance between pupae of the two ladybird species. And the process of adult emergence from the shell was different for each species; adult *E. pubescens* emerged from a wide suture in the shell but the adult *H. variegata* emerged from a hole in the shell. Duration of pupation (development of the adult state inside the shell) at 25° C, in relative humidity 65 ± 5, with 14 hours daylight and 10 hours of darkness took 72 hours (3 days) for *H. variegata* and 120 hours (5 days) for *E. Pubescens*.

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INTRODUCTION

This research was done in the Khorramabad district of Lorestan province of Iran. Ladybirds are important as agents for biological pest control (Gordon 1985; Sadeghi 1991; Obrycki and Orr 1998; William 2002; Farahi and Sadeghi Namghi 2009). Therefore an investigation in to the morphology and behavior of these useful insects is essential research. This study examined morphology of the pupa and adult emergence from the shell of two species of ladybird *H. variegata* and *E. pubescens*. Ladybird is a species of the genus *Hippodamia*. It is valued as a voracious predator of hazardous aphids (Kontadimas and Stathas 2005). Ladybird *H. variegata* has a wide distribution in the Palearctic region (Krafsur *et al.*, 1996; Obrycki and Orr 1998; Franzmann 2002) and it is a well renowned predator of several species of aphid (Wang *et al.*, 1984) This species of ladybird is well known and is found in abundance in most parts of Iran (Bagheri and Mossadegh 1995; Montazeri and Mossadegh 1995; Moadi and Mossadegh 1995; Jafari and Kamali 2007; Farahi and Sadeghi Namghi 2009; Ansari pour and Shakarami 2011). This species of ladybird is the dominant species in most of the alfalfa fields in the Khorramabad region (Ansari pour 2010). The chrysalis or the pupal stage can be described as the resting stage of insects with a complete metamorphosis (David and Ananthkrishnan 2010). In this stage of insect life it is fixed and immobile, pupation of the last larval stage begins and will continue until it becomes an adult. In terms of shape, color and size, insect pupae have many differences. As regards these differences between the pupae of these genus of ladybird there is to date no comprehensive research. So this study has been done to determine differences of various aspects of ladybird pupa of the species *H. variegata* and *E. pubescens*. As such, it was determined that there was difference between these two species and that differences between the genera *Exochomus* and *Hippodamia* do exist.

MATERIALS AND METHOD

Sample collection

Pupa of *H. variegata* and *E. pubescens* were collected from farms, gardens and urban green spaces of the Khorramabad region from alfalfa leaves, apple trees, peach trees and cypress trees. Each of the samples was kept separately and notes were taken on the exact location of the collection site, the specific plants or trees from which the samples were collected and notes on the samples. When the larvae of these two species of ladybirds are ready for transformation to the pupa stage they attach to leaves of plants, so the pupa were collected as they remained attached to fruit trees, cypress trees and were thus brought to the laboratory for the experiment and placed in to special dishes for breeding. The dishes used for this experiment were cylindrical with 4 cm diameter and 7cm in height, these dishes were covered with a ruche. These dishes containing the developing ladybirds were kept at 25° C, relative humidity of 65 ± 5 % in a condition of 14 hours daylight and 10 hours darkness. They were placed in an incubator under these constant conditions of temperature, humidity and light as described above.

Laboratory breeding

After the insects were brought to the laboratory environment, they were examined daily and any changes to occur were carefully reviewed and carefully documented in a notebook, these reviews continued until the adults emerged from shells. After observation of emergence of adults from shells the exact locations of the ladybirds were examined and noted. And the characteristics of color, size and shape were studied carefully and differences were recorded.

RESULTS

Description of pupa *H. variegata*

Pupa color of *H. variegata* was such that initially, following the larval conversion to pupa they were cream colored and after a few days the

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color changed to pale orange and then became dark orange; there were black spots on the dorsal surface of the body of the pupa and the spots were symmetrically distributed. They were not apparent at emergence but appeared after one day. Average body length of the pupa was 4 mm in eight areas of the Khorramabad region (Table 1). This pupa connects to the leaf at the end of the body. After the adult had left the shell of the pupa, the pupa shell became darker in color. The first day after the larvae became a pupa, if the insect was stimulated it was observed that the pupa raised the front of its body where it was joined to the leaves.

Table 1. Length of body in pupa of *H. variegata* in Khorramabad regions

Site	Coordinates	Pupa length (Mean)	Adult length (Mean)
Abestan	3348'N 4857'E	3.7mm	3.8mm
Darvish abad	3340'N 4861'E	4mm	4mm
Moalem Park	3349'N 4835'E	3.9mm	3.8mm
Kakareza	3372'N 4857'E	4.1mm	4mm
Chaghalvandi	3332'N 4828'E	4.2mm	4.2mm
Ghale sangi	3332'N 4819'E	3.8mm	3.8mm
Chegeni	3346'N 4828'E	4.1mm	3.9mm
Kamalvand	3328'N 4828'E	3.6mm	3.7mm

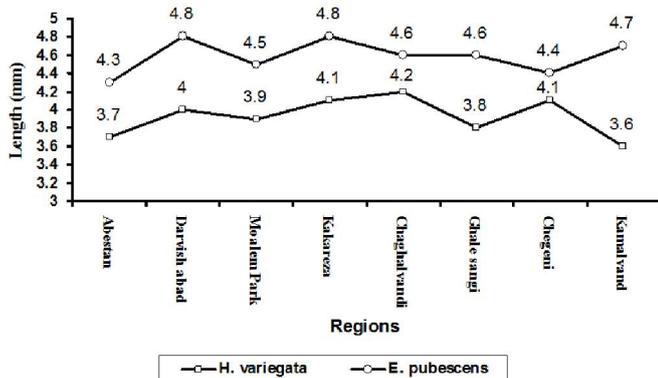


Fig. 1. The difference between pupa length of body in *H. variegata* and *E. pubescens*

Table 2. Length of body in pupa of *E. pubescens* in Khorramabad regions

Site	Coordinates	Pupa length (Mean)	Adult length (Mean)
Abestan	3348'N 4857'E	4.3mm	3.8mm
Darvish abad	3340'N 4861'E	4.8mm	4mm
Moalem Park	3349'N 4835'E	4.5mm	3.8mm
Kakareza	3372'N 4857'E	4.8mm	4mm
Chaghalvandi	3332'N 4828'E	4.6mm	4.2mm
Ghale sangi	3332'N 4819'E	4.6mm	3.8mm
Chegeni	3346'N 4828'E	4.4mm	3.9mm
Kamalvand	3328'N 4828'E	4.7mm	3.7mm

Table 3. Time (days) to become a pupa to adult in various parts of Khorramabad district

	Abestan	Darvish abad	Moalem Park	Kakareza	Chaghalvandi	Ghale sangi	Chegeni	Kamalvand
<i>H. variegata</i>	4.9	5.2	5.4	4.7	5	4.9	5.2	5.3
<i>E. pubescens</i>	3	3.1	3.5	3	3.2	2.9	2.8	2.9

Description of pupa *E. pubescens*

The color of pupae *E. pubescens* on emergence was a pale grayish/brown color. On emergence from the shell the adult was a pale gray/brown color and this remained. The dorsal surface of the pupa shell was covered by many spines (Fig. 4). At the beginning of the pupa stage, the dorsal surface of the pupa shell was completely smooth and without suture but it widened as the adult emerged from the pupa shell. The average body length of the pupae was 4.5 mm. This pupa was connected to the leaf at the end of its body. At the time of pupal stage the insect remained inactive from stimulation.

Behavioral differences in adult emergence from pupa shell

The adult of *H. variegata* emerged from the front pupa shell, for this process the adult made a hole the front part of the shell with its mouthpart and then emerged through that hole. However, adult *E. pubescens* emerged from the shell through a wide suture that exists in the pupal shell (the adult does not pierce the shell of the pupa but exits from a wide suture that already exists). The adult ladybird remained on the shell after emergence until the wings were dry. The color of the elytra did not change.

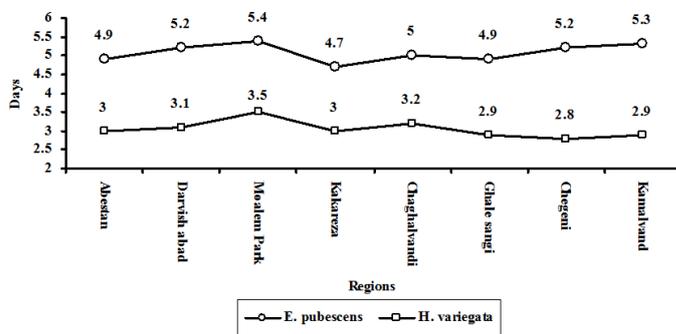


Fig. 2. Time required for the pupae become adult in *H. variegata* and *E. pubescens* in seven regions of Khorramabad district

Time required for become pupa to adult

H. variegata was studied from pupa collected from seven different regions of the Khorramabad district. They were carefully studied during the period when the insect was completely out of the pupa shell (Table 3). Time taken for transformation from pupa to adult took an average of 72 hours (3 days). The times required for pupae to

become adults in each region is given in Figure 2. *E. pubescens* was studied from pupa collected from seven different regions of the Khorramabad district. They were carefully studied during the period when the insect was completely out of the pupa shell (Table 3). The times taken for transformation from pupa to adult took an average of 120 hours (5 days). The times required for pupae to become adults in each region is given in Figure 2.

Pupa size differences

Lengths of pupae in these species are very variable. Length of pupa of *H. variegata* from eight regions of the Khorramabad district were studied, and the results are shown in Table 1. In this table each of lengths are presented as the mean of 20 samples from each region. Length of pupa of *E. pubescens* in eight regions of Khorramabad district were studied and the results are shown in Table 2. In this table each of the lengths are presented as the mean of 20 samples from each region.



Fig. 3. Pupa shell in *Hippodamia variegata*



Fig. 4. Pupa shell in *Exochomus pubescens*

Differences in size between adults emergence from pupa shell

Adult body lengths were studied for *E. pubescens* and *H. variegata*, the results are presented in Tables 1 and 2. In these tables each of the lengths are presented as a mean of 20 samples from each region. Table 2 shows that body lengths of *E. Pubescens* adults (0.6 – 0.8 mm) were smaller than the pupa shell because of the existence in this species of one lamina which separates the body of adult from its pupa shell (Fig. 5).

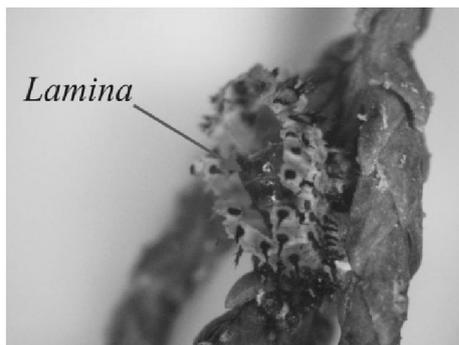


Fig. 5. Pupa shell and lamina in *Exochomus pubescens*

DISCUSSION

There has been no known comprehensive research available on pupa of ladybirds, differences between pupa of two genera or two species and behavior patterns of adult emergence in coccinellidae, therefore none has been cited. Calculations of body length of *H. variegata* and *E. pubescens*, in this research was 3.7 – 4.2mm and 3.7 – 4.2 mm respectively and this is consistent with the research of Sadeghi (1991) and Ansari pour and Shakarami (2011).

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