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**Original Article**

# Biology of *Mylabris impressa stillata* (Baudi, 1878) in a Laboratory Colony (Coleoptera: Meloidae)

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## Abstract

**Background:** In order to study the biology of *Mylabris impressa stillata*, which little is known about, a laboratory colony was established.

**Methods:** In this descriptive study, the laboratory colony was collected from Toyserkan County in Hamedan Province, Iran. To feed the larval instars, a parallel colony of the grasshopper, *Schistocerca gregaria* was set-up. Meloid females lived for several months and laid egg masses with the intervals of about 2 weeks. The first instar larvae, named triungulin, were heavily sclerotized, campodiform, prognathous, and highly mobile. They were fed with fresh eggs of *Schistocerca gregaria* and the pollen paste. This stage was followed by five FG instars which are weakly sclerotized and hypognathous. After a week of being fed, $FG_5$ started digging the soil and subsequently was changed to a coarctate larva which characterized by diapause.

**Results:** We succeeded to break such a diapause in laboratory by chilling at 5º C for four months. Thereafter, the larvae moulted to the next step which is called the Second Grub larvae. SG did not feed and was moulted to pupa in the same moist soil. New adults generally rested for three or four days following emergence, their feeding began at the adult age of a week and sexual behaviour normally appeared at a mean age of about 10 days. The complete life cycle of *Mylabris impressa* took about nine months.

**Conclusion:** Laboratory rearing of blister beetles remains the best tool for ecological and chemical research as well as nuptial gift studies, but very labour intensive and time consuming with low yield.

**Key words:** *Meloidae, Blister beetle, Biology, Laboratory rearing,* Mylabris impressa

## Introduction

Family Meloidae which is a major one in the super family Tenebrionoidea has commonly called as "Blister beetles". Adults of these distinctive terrestrial beetles can be recognized by characters such as soft and rather elongate body and a deflexed head with a narrow neck (1- 4). This family is widespread throughout the world except in New Zealand and Antarctic (1). Diversity is greatest in arid and semiarid regions. The family has currently 120 genera and 3000 species (5). Meloid adults are phytophagous and can be usually found on the plant families such as Asteraceae, Leguminosae, Compositae, Umbliferae and Solanaceae They are grouped in two categories. The first group feeds on the leaves and petals and the second group are mainly pollen feeders. Larvae of leaf feeders are parasites of provisions and immature stages of wild bees, whilst larvae of the other group are parasite of the grasshoppers' eggs. Different species of *Melanoplus* (Insecta: Orthoptera) have been so far recorded as the preferred host of the latter group (6). Apart from subfamily Eliticinae, larvae of all meloids are parasitoids and development is hypermetamorphic with the various instars differing considerably in morphology and behaviour. They differ from those of most other Tenebrionoidea in lacking a mola and urogomphi.

We hereby focus on the tribe Mylabrini which are pollen feeders and whose larvae feed on the grasshoppers' eggs. Considering our routine work

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on cantharidin which is the physiologically active compound of blister beetles, many specimens are required to be dissected and analyzed. Cantharidin is not only a famous aphrodisiac, but a potent blistering agent and has a sound record in medicine and public health. Symptoms of the meloid dermatitis vary from severe but temporary pains to large blisters (7). One hundred and forty two species in 21 genera of blister beetles occur in Iran (8), but only five species are shown to be medically important (Nikbakhtzadeh, unpublished data). Meloid dermatitis has been so far recorded from the western and southern Iran; in particular the western province of Hamedan (8).

To interpret the cantharidin analysis data, it is of great importance to know the sexual behaviour and life history of the beetles, because volume of the chemical may vary depending on the sex, age, mating records and feeding conditions. For example, it is well established that in many species of blister beetles, females possess but can not produce cantharidin. During copulation, males transfer large amounts of cantharidin along with sperm to the female. That is a kind of nuptial gift which provide the beetle with chemical protection by passing on cantharidin to the eggs (5, 9). The rearing procedure of meloids is complicated and needs a great deal of work and patience. We used *Mylabris impressa* stillata (Baudi, 1878), which is also one the medically important beetles of Iran in order to establish a laboratory colony. This species has been so far reported from Hamedan, Kermanshah, Isfahan and Chaharmahal & Bakhtiari provinces in western and central Iran (8) and so far no report exists on its rearing in the laboratory.

In the present work a laboratory colony of meloid beetles was established to observe and control their sexual behaviour and provide an accurate basis for cantharidin intraspecific studies.

## Materials and Methods

To rear meloids towards a descriptive study, we basically followed the detailed instructions of Selander (6) and some general rules for insect rearing (10, 11); however some modifications were implemented to fit it for *M. impressa*.

About 80 specimens of *Mylabris impressa* stillata (Baudi, 1878) were manually collected in Toyserkan county, Hamedan Province, in June and July 2005, by inspection while they were sitting on flowers or stems of different wild shrubs of family Asteraceae. Those beetles which were on the plants or fallen on the ground were picked up and placed in small net ported plastic boxes with the dimensions 18×13×6 cm and transferred to the laboratory in Tehran. The flowers of the native habitat along with some vegetation provided for the beetles as the natural food resource. Besides, small plastic Petri dishes (30 mm diameter) of artificial diet were supplied as a complementary food source. Adults of *M. impressa* feed on pollen, so we developed an artificial diet which proved to be acceptable to the adult specimens. To prepare the diet, water droplets were added to 1 g honey, 1 g sucrose and 3 g of pollen granules to form a pollen paste. Packages (200 g) of pollen granules (Granovita ®) were supplied by Gesundkostwerk Gmbh, Lüneburg, Germany.

Until this step, fourteen beetles had been died during the transport and the first week of laboratory maintenance, but the rest of specimens were transferred into Plexiglas screened cages (50×50×50 cm). Sixty of beetles were picked-up, grouped in three and each group were kept in the Plexiglas cage. The balanced sex ratio was relatively maintained in cages. All rearing efforts were done in a full automatic environmental chamber. A constant temperature of 28º C, a relative humidity (RH) of 40-60% and a daily photoperiod similar to that of the natural habitats (14L: 10D) were implemented. The flowers and other plant materials, offered in bouquet, with the stem in 1% sucrose-water solution to keep them fresh for a longer time at the high temperature of the chamber. Fresh food was given, since meloids were capable of obtaining all their required water from their food. All adults were exactly counted per sex and the cages numbered with date, not to oversight any individual. Natural condition of oviposition was roughly provided by small plastic

boxes (10×10×5 cm), filled with lightly packed moist silica sand.

Eggs lifted carefully by a very fine brush (Pelikan, No.2) and dropped into clean glass vials (8 mm ID×50 mm). All glassware were well washed and then disinfected by 10% Extran MA 04 (Merck, Germany). All vials were marked from outside with a label including data such as date of oviposition and a serial number. Eggs were incubated at 28º C and 100% RH in darkness. To maintain 100% RH, eggs were placed in a vacuum desiccator jar (21 cm ID). The heavy glass lid of the jar fitted tightly except for a 7 mm opening at the top of the lid. The clouded unfertilized and the damaged eggs which developed fungal growth were removed as soon as they were observed. The same environmental conditions as eggs were applied for triungulins, except for a daily temperature cycle with an amplitude of 7º C. As soil for $FG_5$, a mixture of 1 part fine loam and two parts of the water moistured silica sand (10% V) was used.

In order to provide the larval stages of meloids with the fresh eggs of grasshoppers, a large culture of *Schistocerca gregaria* established. 50 specimens (sex ratio 1:1) of a wild population of *S. gregaria* was collected in the southern province of Boushehr and brought to the laboratory in Tehran using cages measuring 40×30×30 cm made from wood, equipped with two screened port. The cages were placed in the same climate chamber as blister beetles, so the same environmental factors implemented, except for the light intensity which regulated at about 750-1000 Lux. The source of light was installed nearby the cage to provide them with both light and enough heating. On the other hand, some little shady areas were obtained where the insects could mate in privacy. Eggs were kept at the same temperature as adult on a moist sand bed, containing 15-20% water. To feed grasshoppers, fresh leaves of Flamenettle (*Coleus blumei*) were offered. A dry food diet such as wheat germ, mixed with the vitamin (Weizenkeime, Schapfenmühle Gmbh and Co., Ulm, Germany) was supplied to provide them with a source of protein.

## Results

Females either oviposit on the floor sheet or the egg masses are laid on the plant materials. Characteristically, the eggs, produced at a given oviposition are deposited in a compact mass. The number of egg per mass varies with body size, but is usually 30 to 40. Adult females are alive for several months and produce egg masses periodically. Egg masses are pale yellow in colour and weakly adherent to each other, relatively large and easily recognized without magnification. In 28º C, 100% RH and total darkness, eggs usually hatch within two weeks.

The first instar larvae are heavily sclerotized, campodiform, prognathous, and highly mobile. They are often referred to as triungulins. Newly emerged triungulin larvae are strongly gregarious and tend to be quiescent. Triungulins were fed with fresh eggs of *S. gregaria* and the pollen paste. In the side (parallel) colony of locusts, female *Schistocerca* lay egg batches inside a sand surface in a depth of about 7-10 cm. Eggs were stored in a place with a constant temperature of about 27-30° C with enough relative humidity. If there is too dry air conditions, eggs will die soon. They have the size of rice grains and yellowish in colour. A frothy secretion over the eggs hardens to form an egg pod. Every female lays 5-7 egg pods with an interval of six days between each pod, every pod comprises of 40-80 eggs. In such a condition, *S. gregaria* needs a developmental duration of approximately 2 months from egg to the adult stage. In order to minimize mortality, not more than 50 individuals were kept in a cage. Grasshoppers' eggs were freed from the pods and offered in loose form. 10 eggs of *Schistocerca* would be enough for a larva of *M. impressa*. This instar is followed by five grub-like, weakly sclerotized and hypognathous scarabaeiform instars referred to as feeding grubs (FG). In 28º C, larvae reached the first grub 5 ($FG_5$) instar in 4-6 weeks. $FG_5$ was fed for about a week by the same diet (eggs of *S. gregaria* and the pollen paste) and then freed on the surface of a soil filled plastic box (10×10×5 cm). It usually

starts digging the soil which takes about a day to be completed. On completing feeding, $FG_5$ prepares a cell in the soil apart from the grasshopper egg pod on which it fed and typically becomes, at ecdysis, a heavily sclorotized and immobile coarctate larva (C). This larva enters diapause and passes the winter or other unfavourable period of the year in the coarctate phase. When near the beginning of the next season of adult activity, the larva undergoes ecdysis, it again becomes grublike in form (second grub phase, SG). This phase, which entails only one instar is followed shortly by pupation and adult emergence. Life stages of the first and the second generations of our laboratory-reared colony of *M. impressa stillata* have respectively been summarized in Table 1 and 2. Feeding begins at the adult age of a week and sexual behaviour normally appears at a mean age of about 10 d. The minimum age of males at the first courtship is 6 d. As a general rule the deposition of the meconium occurs a few hours to a day before feeding begins.

**Table 1:** The life cycle of the first generation of *Mylabris impressa stillata* in the laboratory (2005-6)

| June | July | Aug | Sep | Oct | Nov | Dec | Jan | Feb |
|---|---|---|---|---|---|---|---|---|
| Adult* | Adult | Adult | Adult | | | | | |
| Egg | Egg | Egg | | | | | | |
| | Triungulin | Triungulin | Triungulin | | | | | |
| | $FG_1$ | $FG_1$ | $FG_1$ | | | | | |
| | $FG_2$ | $FG_2$ | $FG_2$ | | | | | |
| | $FG_3$ | $FG_3$ | $FG_3$ | | | | | |
| | | $FG_4$ | $FG_4$ | $FG_4$ | | | | |
| | | $FG_5$ | $FG_5$ | $FG_5$ | | | | |
| | | | | C | C | C | C | |
| | | | | | | SG | SG | |
| | | | | | | PP | PP | |
| | | | | | | | P | P |
| | | | | | | | | Adult** |

* These adults were directly collected from the field

** $F_1$

Abbreviations: FG: first grub, C: coarctate, SG: second grub, PP: prepupa, P: pupa

**Table 2:** The life cycle of the second generation of *Mylabris impressa stillata* in the laboratory (2006-2007)

| Feb | March | Apr | May | June | July | Aug | Sept | Oct |
|---|---|---|---|---|---|---|---|---|
| Adult* | Adult | Adult | | | | | | |
| Egg | Egg | Egg | | | | | | |
| | Triungulin | Triungulin | Triungulin | | | | | |
| | $FG_1$ | $FG_1$ | $FG_1$ | | | | | |
| | $FG_2$ | $FG_2$ | $FG_2$ | | | | | |
| | $FG_3$ | $FG_3$ | $FG_3$ | | | | | |
| | | $FG_4$ | $FG_4$ | $FG_4$ | | | | |
| | | $FG_5$ | $FG_5$ | $FG_5$ | | | | |
| | | | | C | C | C | C | |
| | | | | | | SG | SG | |
| | | | | | | PP | PP | |
| | | | | | | | P | P |
| | | | | | | | | Adult** |

* This adults were offspring of the first laboratory-reared generation ($F_1$)

** $F_2$

Abbreviations: FG: first grub, C: coarctate, SG: second grub, PP: prepupa, P: pupa

## Discussion

The mentioned procedure of blister beetle rearing has been designed for individual rearing of larvae and therefore work-intensive, because no successful attempt at mass rearing of larvae is so far reported. The reason is mainly the fact that the larvae, once they have begun to feed are cannibalistic (6).

In order to control the courtship and copulating, newly emerged adults were immediately separated based on the sex and were kept in different cages. It is extremely important to chemical ecologist; because the ground level of many chemicals can be intervened by sexual behaviour, e.g. courtship and copulation and so baffle the chemical measurements.

Rearing of blister beetles has ever been a very delicate and complex system which needs too much efforts and time. To now, only a few Nearctic species have been successfully cultured and other efforts have failed to set up a colony of other species (6). Some of the difficulties in setting up of a laboratory colony of blister beetles deal with their univoltinism. Besides, they indicate hypermetamorphosis in which each larval stage needs specific food requirements. There may be several steps of quiescence and diapause during the larval and pupal stages. For example, the diapause of the coarctate larvae was even occurred in the laboratory and was only broken by chilling of C larvae at 5º C for four months until they were changed to the Second Grub larvae (SG). After breaking the diapause, SG did not feed and was moulted to pupa in the same moist soil. If diapause is not broken, as it normally occurs among field populations of a temperate region, the coarctate larva does not moult to SG in less than 6 months. Eggs, some larval instars, prepupae and pupa are laid beneath the soil surface and very sensitive to the food and environmental conditions. In nature, females normally oviposit in burrows which they excavate with the mandibles and legs and subsequently refill (12-19).

Regarding their parasitism on either wasps or grasshoppers, a colony of one of these two must be first established to provide a reliable feeding source for the larval instars. By the way, phytophagous adults usually have host specificity and supplying their required plants in captivity is rather hard.

The complete life cycle (from adult to adult) of *Mylabris impressa* takes about nine months and the adults are usually alive in captivity for 3-4 months. In hot arid areas, such as most parts of Iran, it has been found that a huge number of aggregated beetles tend to remain on or near the ground, under dense foliage for the greater part of the day (12, 20) presumably because the adults indicate a periodical activity in response largely to varying conditions of heat and humidity. The aggregation property among individuals is assumed to be induced by cantharidin which acts as an aggregation (13, 14) and a close range pheromone (21).

We continued to rear the beetles for two generations (2005-2007), but later stopped it due to the maintenance costs in late 2007 when no further demand was predicted. While only a few species of blister beetles have been so far reared in the lab, we could establish another species of blister beetles to study its biology. Laboratory rearing remains the best tool for ecological and chemical research as well as nuptial gift studies, but this is a low-yield system with delicate equilibrium. Even a well-settled colony needs a strong scientific justification to be maintained.

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