Mitt. Münch. Ent. Ges.	**95**	11-18	München, 31.01.2006	ISSN 0340-4943

Description of early stages and laboratory breeding of *Olepa schleini* WITT et al., 2005 (Lepidoptera, Arctiidae)

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Abstract

The early stages of *Olepa schleini* WITT et al., 2005 (Lepidoptera, Arctiidae) are described and illustrated. In addition, the results of successful ex ovo breeding are presented. The eggs are round and light yellow. The average clutch size is 148 eggs. The first three of the six larval instars are of a light greenish colour with sub-dorsal black maculae that increase in size over the time. After the third moulting, larvae are distinctively blackish-brown with only discrete yellow lines that vanish in the last, fox-red coloured instar. After 22 days, larvae pupated in a strong cocoon. The hair (setae) of larvae of the last three stages and those of the cocoon are strongly allergenic. Under natural conditions, a partial third generation was observed while in standard insectary conditions the multiplication was uninterrupted. The young greenish larvae were sensitive to starvation while the brownish stages suffered severe losses from overcrowding. Bacterial and microsporidial (protozoa) infections were common.

Introduction

Intensive collecting was conducted from 1986-2004 all over Israel to achieve the purposes of the Israeli-German project for the study of the Israeli Lepidoptera fauna. The project was a joint effort of The Hebrew University, Tel Aviv University, the Nature Reserves and National Parks Authority of Israel, the Zoologische Staatssammlung, Munich, and Museum Witt, Munich, Germany. *Olepa schleini* was discovered at a relatively late stage of the project (WITT et al. 2005) and for three years its early stages were unknown. Generally, little was published about breeding, the early stages and biology of the genus *Olepa*. *Ricinus* (Euphorbiaceae) as host plant of *Olepa ricini* (FABRICIUS, 1775) is mentioned by FABRICIUS himself (1775; 1781) with the expression 'Habitat in Indiae orientalis ricino'. MOORE (1883) lists *Notonia* (Asteraceae), *Fagraea* (Loganiaceae) and *Dahlia* (Asteraceae) as host-plants for *Olepa ocellifera* (WALKER, 1855). The larvae of *Olepa duboisi* Orhant, 1986 have been reared on dandelion ('pissenlit', *Taraxacum*), plantain (*Plantago*), and roots of endives ('racines de chicorée de Bruxelles', *Lactuca*) (ORHANT, 1986). However, some details of the biology of *O. schleini* and its behaviour were succinctly described yet 2500 years ago in the book of Jonah (HAUSMANN & MÜLLER 2006).

Description of the early stages of *Olepa schleini*

The eggs are round and light yellow. They are typically deposited in clutches of about 130 eggs or more, arranged in a single layer and in parallel tight rows.

Generally, the first three of the six larval instars are of a light greenish colour with sub-dorsal black maculae that gradually become bigger. After the third moulting, larvae are distinctively blackish-brown with only discrete yellow lines that vanish in the last instar.

***L1*:** A freshly hatched larva is greenish white, covered with thin pale setae, each abdominal segment has a large black sub-dorsal spot on each side and the head is black.

***L2 & L3*:** The second and third stages have stronger and darker setae and their black spots increase in size. The medio-dorsal and lateral lines remain greenish; however, in the third stage, the sub-dorsal area becomes entirely black.

***L4*:** Fourth stage larvae are almost entirely black, with only narrow yellow dorsal and lateral lines.

***L5*:** In the fifth stage, the larvae become more reddish brown, with unchanged dorsal line, thinner lateral lines and pale brown 'hair'.

***L6*:** The appearance of fully grown larvae is totally different. The cuticle is entirely black without longitudinal lines and, except for the naked inter-segmental and underside areas, it is covered with bundles of long fox-red to pale brown 'hair'. The prolegs and thoracic legs are reddish-brown and the head is shiny black.

Pupation takes place in a cocoon made of white silk and the highly irritant setae of the full grown larvae are incorporated in it. Within a few days, the cocoon darkens and becomes medium brown.

The pupa, a typical pupa obtecta, is characteristic for Arctiinae. The colour is shiny black and the form is elongate-oval, slightly flattened in the head-region. The smooth hairless pupal skin is comparatively thick (as usual in Arctiidae).

Plate 1: Fig. 1-6 The early stages of *Olepa schleini* WITT et al., 2005 from egg to third instar larva. **1,** Eggs. **2,** Fresh hatched larvae. **3,** L1 larvae (1 day old). **4,** L2 larvae (5 days old). **5,** L3 larvae (9 days old). **6,** L3 larva (12 days old)

Breeding of *Olepa schleini*

The colony of *Olepa schleini* was established with eggs collected from the natural host-plant, a *Ricinus communis* L. (Euphorbiaceae) tree in Nahal Gerar on the Coastal Plain of Israel in mid-September 2001. Groups of the immature stages of the parental (P) and the following (F1) generation were reared under outdoor conditions in the Zoological Garden of Tel Aviv University, and other groups were reared in insectaries in the Hebrew University, Jerusalem. The Tel Aviv colony was kept under a canvas construction in the shadow of *Ricinus* trees. The laboratory colony was maintained at 26 ± 10C, 60% relative humidity (RH), and a photoperiod of 17:7h (L:D). Rearing cages were plastic/ carton boxes of sizes that suited the growing larvae. They were covered with stretched artificial-silk stocking, gauze, or fly mesh.

The breeding protocol of a F1 generation in Tel Aviv was as follows.

Eclosion of adult *Olepa schleini* began typically at sunset and continued for about three hours. Immediately afterwards, pairs of P generation males and females were each placed for egg laying in a paper box of 20x15x30cm, containing some tissue paper.

Adult copulation took place on the night of eclosion or a night later and oviposition began two to three nights later and continued for three nights. The total number of eggs per female varied from 98 to 236 eggs with an average of 148 (n = 150) and the first batch of eggs contained normally 80% or more of the totally laid eggs. Virgin females laid only few or no eggs. Females died one or two days after oviposition and adult males lived six days (5-7) on average. Like most Arctiidae, adults did not feed (slices of apple) but water from cotton swabs was readily accepted.

The eggs were laid and firmly attached in patches mostly on tissue paper but also on other kinds of available surface. Groups of about 100 eggs were each transferred with the substrate to a 15cm diameter transparent plastic Petri dish, containing a single *Ricinus* leaf that was replaced daily.

After three days, the eggs darkened and the heads of larvae were visible through the shell. Hatching was on the 4th day and initially the larvae did not move from the substrate to a nearby *Ricinus* leaf and fed only on the egg shells. During their second day, the L1 larvae moved to the leaf and ate in dense groups that were attached to the leaf with a silky web.

After moulting, they were transferred in batches of about 50 specimens to plastic boxes (10x20x8cm) that had several layers of tissue paper at the bottom and a gauze cover that allowed aeration preventing water condensation. Food leaves and the tissue paper were changed daily.

L2 and L3 larvae were kept in groups of 30 and 15, respectively, in the same containers under the same conditions.

L4 and L5 larvae were kept in groups of 15 specimens in 30x30x20cm plastic containers with a 3cm layer of untreated pinewood chips at the bottom and a cover of common plastic fly mesh. The food, small *Ricinus* branches, and the wood chips were changed daily.

L6 larvae in groups of 15 were kept in heavy carton boxes 70x50x20cm, re-enforced with metal fly mesh, as commonly used for shipping laboratory rodents. These boxes had a wood chip layer at the bottom. The larvae were allowed to feed on large *Ricinus* branches that were changed daily. The wood chips were not changed and, generally, these boxes were handled as little as possible to avoid unnecessary disturbance. For several hours up to one day before pupation, the larvae were moving energetically and, in smaller cages, they, thus, interrupted other larvae that were already spinning cocoons or pupating.

Pupation took place 20-26 days after eclosion. Cocoons in clusters had to be separated after some days when pupae had hardened. The separation often damaged the exit point and separated cocoons often yielded crippled adults. Good results were obtained when the hardened pupae had been taken out of their cocoons and kept on fan shaped folded filter paper with 1cm troughs.

For the eclosion, the cocoons were removed from the breeding boxes and placed on the bottom of carton boxes with a minimal size of 80x50x50cm that were covered with gauze. Fan shaped filter papers with pupae were leaned at an angle of 30° on the walls. The size of boxes was important since adults were damaged when they flew in smaller boxes. Eclosion occurred in the early evening and night. The chronological timing was also retained under constant light but then the adults did not fly and therefore remained in good condition. With a 24 hr light regime, it was also possible to keep up to 500 pupae in a single carton while in natural light conditions the maximal number was not more than a dozen per box. The sex ratio of young adults was 0.75 females : males in both rearing places.

Under natural conditions, in the Zoological Garden Tel Aviv, *Olepa schleini* had two full generations in the hot season and a partial development of a third one occurred in the late autumn. Under standard

insectary conditions there were continuous generations. Diapause in the pupae was induced in the insectary, in the late autumn, by a natural light regime. Pupae survived temperatures as low as -5°C while eggs and larvae did not survive temperatures below 0° C.

After several days in temperature below 10°C, larvae stopped feeding and died, young stages sooner than older ones.

Nicotiana glauca GRAHAM (Solanaceae) can be used as alternative food to *Ricinus*. This toxic plant of South American origin has become common in Israel. Larvae did not feed on the following native Israeli plants: *Acacia raddiana* SAVI (Mimosaceae), *Amygdalus communis* L. (Rosaceae), *Atriplex halimus L. (Chenopodiaceae), Capparis spinosa L. (Capparaceae), Crataegus azarolus* L. (Rosaceae), *Salix acmophylla* BOISS (Salicaceae), *Malva nicaeensis* ALL. (Malvaceae), *Ochradenus baccatus* DEL. (Resedaceae), *Populus euphratica* OLIV. (Salicaceae), *Prosopis farcta* BANKS & SOL. (Mimosaceae), *Pistacia palestina* BOISS. (Anacardiaceae), *Quercus ithaburensis* DECNE. (Fagaceae), *Rubus tomentosus* BORKH. (Rosaceae) *Trifolium resupinatum* L. (Papilionaceae) and several Gramineae species.

Larvae reared from eggs laid in early June pupated within the next three weeks and adults emerged from July to October with a pronounced peak in August-September. Only a small proportion (12.5%) of the larvae hatching from early September eggs developed to adults in the late autumn of the same year. The rest of them diapaused as pupae and the adults emerged from May to September with a peak in July and August.

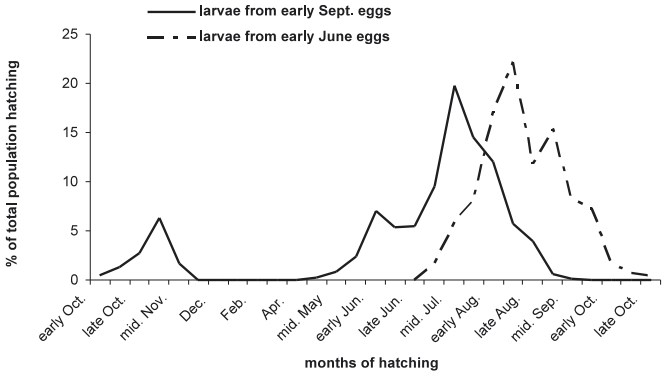

Diagram 1: Phenology of hatching adults in outdoor rearing.

Release of bred *Olepa schleini* into nature

Large amounts of *Olepa schleini* adults as well as larval stages were released in the vicinity of existing colonies from which material was collected for breeding experiments and museum collections. The released *O. schleini* by far outnumbered the specimens which had been collected in nature. All the releasing experiments were successful. Nevertheless these artificially established populations showed huge fluctuations in the following years as also the natural colonies did (MÜLLER et al. 2006). It is remarkable that collecting of large numbers of caterpillars from the accidentally established colony in the Zoological Garden of Tel Aviv University over several years did not seem to influence this colony.

Additional comments

Larvae of all stages fed readily on freshly cut leaves, on leaves and branches that had been kept in plastic sacks for several days in a cold room at 5°C and also on branches that were maintained with their stems in water for up to a week.

Plant material and the layer at the bottom of the breeding containers must be routinely changed on a daily basis. In addition, all breeding containers have to be washed daily and wiped with 70% Ethanol. Cages

with signs of infection like: diarrhea, apathy of larvae, loss of larval turgor, lack of feeding, or unexplained mortality, should be destroyed immediately or isolated in a different room (SINGH & MOORE 1985).

The brownish L4-L6 stages that feed solitarily in nature were found to be very sensitive to crowding. Crowding, high humidity with condensation in the cages or dirt can cause quick and total mortality. New disposable gloves should be used and instruments (forceps, brushes, etc.) must be properly cleaned with Ethanol to avoid transmission of pathogens from cage to cage. Cage covers should be kept with their original cages after cleaning and carton boxes should be used only once. Identified pathogens were bacteria and microsporidians.

Microsporidia are the most important protozoan pathogens of both beneficial (HAYASAKA et al. 1993; HENRY et al. 1978) and pest insects (BROOKS 1988). They can become a serious problem in insect colonies (WEISER 1961). These intracellular parasites can be transmitted horizontally by copulation, excretion, food consumption or vertically by infected eggs (WEIDNER 1989). The microsporidial species that infected *Olepa schleini* was not identified and its mode of transmission was not investigated. Microsporidia were also found in larvae from the field and it is possible that the pathogen was introduced from the field into the laboratory colony. The parasites were not eliminated by sanitation methods like sterilization of all the equipment with Ethanol, bleach and UV radiation. Some infected colonies were successfully treated with an aqueous solution of 3% Benomyl (HSIAO & HSIAO 1973; BROOKS et al. 1978; BRIESE & MILNER 1986). The solution was sprayed on leaves that were dried and offered to infected larvae.

The L1-L3greenish stages were very vulnerable while moulting. The first and second instars moulted in dense groups on the leaves while they were attached by silky webs. Transfer of moulting larvae to another leaf resulted in huge mortality. At this time, the leaves were cleaned of feces with a small paint brush and placed together with fresh leaves. At other times, the small larvae were moved with a paint brush from leaf to leaf.

Later the larvae moulted on the cover of the boxes and maintenance of the colony was unproblematic. Nevertheless, the bigger larvae should not be disturbed at this phase.

The greenish stages were rather sensitive to starvation while the brownish stages fed readily on twigs, seeds and bark of small branches after the leaves had been consumed. Cannibalism on any of the larval stages was not observed but in crowded conditions or under starvation brown larval stages fed on newly formed pupae.

The larvae of *O. schleini* are a serious health hazard. From the fourth instar onwards, the larvae carry urticating hairs which can cause strong allergic reactions of skin, eyes and sometimes the respiratory mucous membranes. The strong allergic reactions can last for several days. Anaphylactic shock situations known from Thaumetopoeidae larvae (ARLIAN 2002**)** were not seen. Precautions should be taken with contaminated cages any kind of equipment and laboratory clothes. It is recommended to constantly use disposable gloves and to clean the equipment and the laboratory every day. Particularly L6 larvae and cocoons should be handled under a hood. In our case, escaped L6 larvae pupated in any kind of dark places up to 3.5m just below the ceiling and one specimen traveled two floors up to a distance of about 250m.

Zusammenfassung

Die Präimaginalstadien von *Olepa schleini* WITT et al., 2005 (Lepidoptera, Arctiidae) werden beschrieben und abgebildet. Desweiteren werden die Ergebnisse einer erfolgreichen Eizucht vorgestellt. Die runden, hellgelben Eier werden in Gelegen von durchschnittlich 148 Eier abgelegt. Die ersten drei der sechs larvalen Häutungsstadien zeichnen sich durch hellgrüne Färbung aus, die subdorsalen dunklen Makel vergrößern sich im Laufe der Entwicklung. Nach der dritten Häutung wechselt die Grundfärbung nach schwarzbraun, mit schmalen gelben Längslinien, die im letzten, fuchsrot behaarten Häutungsstadium verschwinden. Nach 22 Tagen verpuppen sich die Raupen in einem kräftigen Kokon. Die Behaarung (setae) der Raupen und die in den Kokon eingewobenen Haare rufen starke allergische Reaktionen hervor. Unter natürlichen Bedingungen wurde eine unvollständige dritte Generation beobachtet, während im Insectarium unter Standard-Laborbedingungen ständig neue Generationen über das ganze Jahr hinweg hervorgebracht werden konnten. Die jungen, grünen Larvenstadien zeigten sich empfindlich gegen Futtermangel, während die älteren braunen Stadien bei zu enger Haltung deutliche Verluste erlitten, vor allem durch Bakterien- und Microsporidia-infektionen (Protozoa).

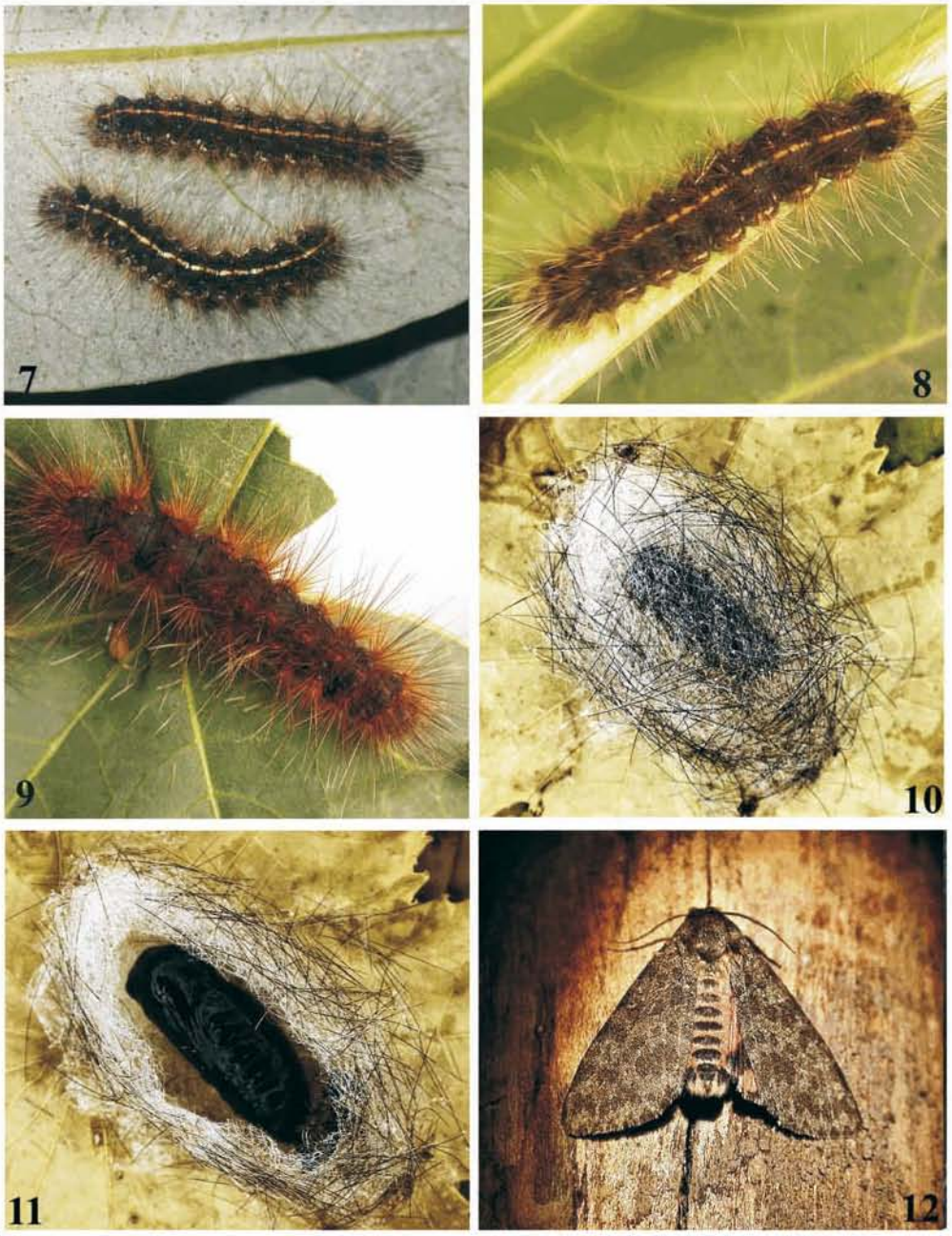

Plate 2, Figs 7-12. The early stages of *Olepa schleini* WITT et al., 2005 from fourth instar larva to adult. **7,** L4 larvae (12 days old). **8,** L5 larva (17 days old). **9,** L6 larva (22 days old). **10,** Cocoon (fresh). **11,** Cocoon opened with pupa. **12**. Adult female.

Acknowledgements

We are grateful to the Israeli Nature and Parks Authority, especially Dr. R. ORTAL (NPA), for the collecting permits and to the staff of the NPA- regional rangers and the regional & district ecologists for help with the field work. We thank all the technicians and students who helped to maintain the colonies. This study would not have been possible without the generous help of Prof. Y. SCHLEIN, Hebrew University, Medical School. We are grateful to Dr. L. SCHNUR for revising the manuscript.

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