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BIOLOGY OF THE QUEEN OF SPAIN FRITILLARY, *ISSORIA LATHONIA* (*LEPIDOPTERA: NYMPHALIDAE*)

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ABSTRACT

The biology and the life cycle of *Issoria lathonia* (Nymphalidae) (Linnaeus 1758) on its host plant, *Viola tricolor* L. (Violaceae), are described from laboratory studies. In the laboratory eggs are laid singly on the host plant leaves as well as on the surfaces of plastic screen cages. Newly hatched larvae aggregate and feed on the host plant leaves. Later instars disperse on the plant and continue to feed on leaves and flowers. Head capsule widths, and weight and size measurements show that larvae develop through 5 instars. The larvae crawl off the host plant and pupate off the host. The life cycle from egg to adult requires 23-31 d at 26°C, and 16:8 (L:D) photoperiod in the laboratory. The butterfly has been reared continuously in the laboratory for about 2 years.

Key Words: *Issoria lathonia*, Nymphalidae, Argynnini, *Viola tricolor*

RESUMEN

Se describe la biología y el ciclo de vida de *Issoria lathonia* (Linnaeus, 1758) sobre su planta hospedera, *Viola tricolor* L. (Violaceae) basado sobre estudios de laboratorio. En el laboratorio los huevos están puestos individualmente sobre las hojas de la planta hospedera igual como sobre la superficie de la tela plástica de las jaulas. Las larvas recién nacidas se agregan y se alimentan sobre las hojas de la planta hospedero. Los instares posteriores se dispersan sobre la planta y continúan su alimentación sobre las hojas y flores. Las medidas de la anchura de las cápsulas de las cabezas, el peso y el tamaño muestran que las larvas pasan por cinco instares. Las larvas caminan fuera de la planta hospedero y empupan separadas del hospedero. El ciclo de vida desde el huevo hasta el adulto requiere 23-31 días a los 26°C y un fotoperíodo de 16:8 (L:D) [16 horas de luz y 8 horas de obscuridad] en el laboratorio. La mariposa ha sido criada continuamente en el laboratorio por aproximadamente 2 años.

The nymphalid genus *Issoria* Hübner 1819 includes 97 species distributed throughout the Palaearctic region and in parts of Africa, including the *I. eugenia* group, *I. lathonia* group and the African *I. smaragdifera* group. *Issoria lathonia* L. is commonly known as the queen of Spain fritillary across Europe.

Simonsen (2006 a, b) suggested from morphological studies that two of the African species (*I. hanningtoni* and *I. baumanni*) may not belong to *Issoria*, but molecular data (Simonsen et al. 2006), indicates that the African species (including *I. smaragdifera*) form a clade within *Issoria*.

Issoria spp. in the tribe Argynnini (Fritillaries) have been studied with respect to female abdominal scent organs (Urbahn 1913), glands, muscles and genitalia (Simonsen 2006c), genitalic morphology and function (Shirôzu & Yamamoto 1959; Arnold & Fisher 1977; Ockenfels et al. 1998; Simonsen 2006a,b), classification, and host plant records and oviposition sites (Oliveira & Freitas 1991), and host plant relationships (Ackery 1988). There are few studies of the immature stages and life cycle of *Issoria* spp., and none for *Issoria lathonia* L. the queen of Spain fritillary.

The adults of the queen of Spain fritillary are characterized by a strong orange and black coloring of the upper sides of the wings (Baytas, 2007; Tolman & Lewington 1997). The shape of the wings differs from other fritillaries in being slightly angular. The undersides of the hindwings have large, oblong silvery patches. The line with yellow dots (2 bigger and 1-2 smaller) in the apical corners of upper side forewings is very common. Adult males and females look alike. The queen of Spain occurs in the Canary Islands, Madeira, North Africa, Southern, Central and Eastern Europe, Southern Sweden, Finland, and Turkey. The species generally is active from Feb to late autumn, with 2 to 3 generations. It may hibernate in the egg, larval, pupal, or adult stage (Tolman & Lewington 1997). *Viola tricolor* L. and *Viola arvensis* Murr. (Violaceae) are common larval host plants, but Simonsen (2006 a, b) found larvae feeding on *Anchusa* sp., *Ribus* sp., and *Onobrychis* sp. in Europe. The eggs are laid singly on leaves of the host plants or nearby weeds.

The aims in this paper are to describe the biology and immature stages of *Issoria lathonia* L. feeding on *Viola tricolor* L. in the laboratory.

Materials and Methods

During May and Jun 2006, *Issoria lathonia* adults (n = 35) were captured in the vicinity of Canakkale, Turkey, at about 325 m above sea level. Adults were kept in screen cages (60 × 60 × 60 cm) with *Viola tricolor* host plants. A number of flowering plants (*Lantana camara* L., *Dianthus* sp., and *Carduus* sp.) were provided in the adult rearing cages along with 10% honey solution or Orange Punch Gatorade® dispensed on cotton balls to provide food for the adults. Eggs were laid on the host plant leaves as well as on the screen cage, and they were removed daily, counted, and kept in a Petri dish on moist filter paper. Hatching larvae were allowed to feed on freshly cut host plant material obtained as potted plants from local garden shops as needed. Larval food was changed daily by transferring all larvae to new plants. Pupae were harvested daily and transferred to a new cage with a potted host plant and adult food source. The colony was maintained under controlled laboratory conditions at 26 ± 1°C, 60% RH and 16:8 (L:D) photoperiod. The number of instars was determined from data collected from 15 larvae examined each day. Shed larval head capsules were collected, measured, and preserved in 70% ethyl alcohol. Larvae were weighted and their length measured daily for the 15 individuals. All biological stages of *Issoria lathonia* were examined and photographed with an Olympus C7070 wide zoon camera attached to an Olympus SZX9 binocular stereo zoom microscope. The LSD test at 0.05 level of significance was used to determine separation and significance of means (SAS 1990).

Results

Females laid their eggs singly, either on the host plant or on the mesh screen of the cage. The eggs are elliptical, pale yellowish, conical, and flattened at the top, with 20 to 22 longitudinal ridges (Fig. 1A). They are about 0.38 ± 0.01 mm in length and 0.31 ± 0.01 mm in diameter (n = 17). The color of the eggs changed from pale yellow to brownish

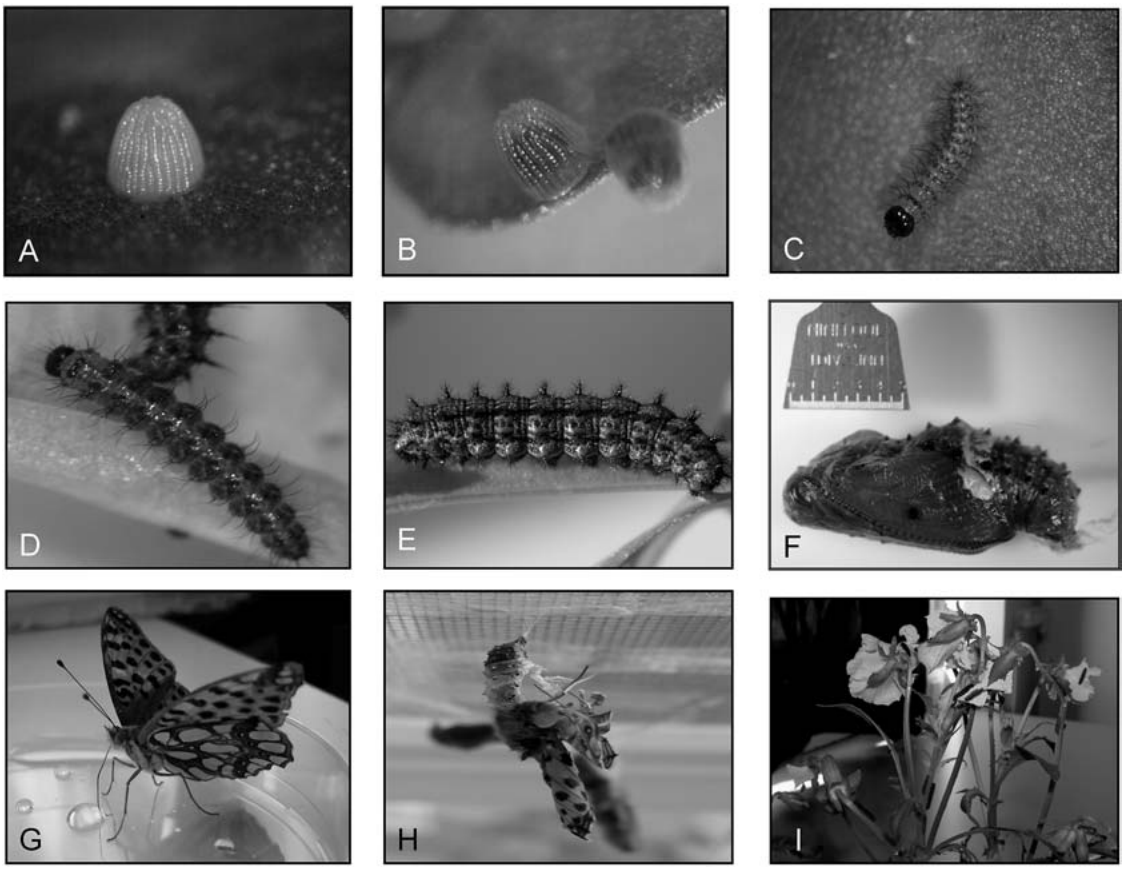

Fig. 1. Immature stages of *Issoria lathonia*. (A) An egg; (B) An egg about to hatch; (C) First instar; (D) Second instar; (E) Fifth instar, (F) Lateral view of pupa; (G) Adult feeds on honey solution (H) Unsuccessful adult emerging (I) Laboratory colony on *Viola tricolor*.

black (Fig. 1B), after about 3-4 d as the mandibles and head of the larvae became visible through the chorion. The eggs hatch in 3-4 d at 26°C.

We determined that there are 5 instars based upon weight, length, and head capsule measurements (Table 1). The duration of time spent in the egg stage, in each of the 5 instars, and the pupal stage are shown in Table 2.

The first instar body is initially translucent yellow but changes to pale dark yellow after feeding. There are long setae over the body (Fig. 1C). The head is dark brown to black and the mouthparts are dark yellow. The legs and prolegs are translucent yellow and tarsal segments are dark brown to black. First instars aggregate and prefer to feed on flower parts of the host plant. Second instars are gray-brown in color with gray lateral bands (Fig. 1D). Each segment contains a row of long spines. The head is black and the mouthparts are dark brown black. Longitudinal dorsal and subdorsal bands are evident. The thoracic legs are brown with the tarsal claws darkened. Third instars are similar in appearance to second instars, but each segment contains a row of short, branching spines. The head is black with light brown eyes. Third instars no longer aggregate, but distribute themselves over the plant. The fourth and fifth instars are similar in appearance to each other and to third instars (Fig. 1E). Cream-white patches are clearly evident on the black background of the body of fourth and fifth instars. A row of short branching spines are evident, are orange colored at the base. The legs are black but the prolegs are light brown-orange. Fourth instars consumed a large quantity of host leaves and flowers (Fig. 1I). Mature larvae attached with the cremaster to a supportive surface and remained in a crescent shape about 5-6 h. Then, hanging straight down, they changed within 3-5 minutes into the characteristic pupal shape and appearance. The pupal color is variable to light brown to dark brown-black, but shiny, uniform, and brownish with white dorsal patch resembling a bird-dropping. Pupae are initially very soft and dark brown (Fig. 1F). They have a black patch area over the wings, and the labial palpi and antennae were visible through the cuticle. The pupal abdomen consists of 10 segments, with the 10th bearing the cremaster. Female pupae are 1.50 ± 0.01 mm long and 0.31 ± 0.03 mm wide (dorsoventral measurement in the thoracic region), and weighed an average of 289.4 ± 46 mg (n = 21). Male pupae are 1.70 ± 0.04 mm long, 0.41 ± 0.03 mm wide, and weighed an average 313.4 ± 51 mg (n=26). Females emerged from the pupal stage about a day earlier than males, on average (Table 2).

Adult males and females were similar in appearance (Fig. 1G). The wingspan was 39.2 ± 2.90 mm in females and 40.3 ± 1.98 mm in males (n = 10). Mating pairs often rested quietly together 4-5 h. Females started laying eggs about 2 d after mating. Adults survived in the laboratory about 2 weeks. The duration from egg to adult was 23-31 d at 26°C, 16:8 (L:D) photoperiod. In the laboratory, 38% of pupae failed to become adults (Fig. 1H).

Discussion

This is the first detailed description of the biology of *Issoria lathonia* L. and of the immature stages. The larvae of the queen of Spain fritillary feed on several species in the genus *Viola* in Violacea, but the main food plants are wild pansy (*Viola tricolor* L.) and field pansy (*Viola arvensis* Murr.). These plants are larval food plants for some other nymphalid species, including *Boloria bellona*, *B. selene, Speyeria aphrodite*, *S. atlantis*, *S. cybele*, *Argynnis pandora*, and *A. paphia* (Hesselbarth et al. 1995). *Viola aetolica*, *V. lutea*, *V. biflora*, *V. calcarata*, *V. corsica*, and *V. odorata* have been recorded as larval host plants for *Issoria* spp. (Tolman & Lewington 1997), but larvae refuse to feed on "African violets" (*Saintpaulia* spp.) (Tolman & Lewington 1997). *Issoria lathonia* has strong local migratory habits and is an endangered species (Verovnik 2000; Kotiaho et al. 2005) in many countries. It occurs in a wide variety of habitats where the larval food plants occur.

In our study, *Issoria lathonia* adults mated readily in the laboratory. Availability of the larval host plant in the adult cages seemed not to be crit-

Table 1. Measurements of head capsule, weight, and length of larval *Issoria lathonia* in each instar (mean ± sd, N = 15).

Instar	Head capsule width (mm)	Weight (mg)	Length (mm)
First	0.186 ± 0.00 a	0.02 ± 0.01 a	2.66 ± 0.61 a
Second	0.338 ± 0.03 b	3.52 ± 0.30 b	4.73 ± 0.79 b
Third	0.508 ± 0.02 c	14.42 ± 1.61 c	7.46 ± 0.99 c
Fourth	0.789 ± 0.02 d	63.49 ± 11.05 d	13.80 ± 2.54 d
Fifth	1.231 ± 0.07 e	213.09 ± 48.67 e	21.66 ± 2.92 e
LSD*	0.0289	0.7237	1.2803

*LSD= Fisher's Least Significant Difference between any 2 means. The means with a column followed by a different letter are different from each other ($P \leq 0.05$).

TABLE 2. DEVELOPMENT TIME FOR LABORATORY CULTURE OF *ISSORIA LATHONIA* AT 26°C (MEAN ± SD, $N = 15$).

Stage/instar	Days
Egg	3.70 ± 0.7
First	3.00± 0.6
Second	2.93 ± 1.1
Third	2.53 ± 0.8
Fourth	2.73 ± 0.7
Fifth	4.00 ± 0.5
Pupae	
Female	4.26 ± 0.7
Male	5.30 ± 0.7

ical for adult oviposition. The host plant, *Viola tricolor,* is widely available as an ornamental plant in the local greenhouses and it also can be cultured easily in small pots. During 2 years of rearing the queen of Spain fritillary butterfly we found some evidence of disease, especially in the pupal stage. Further studies are necessary to solve this problem. The migratory status of the butterfly in Turkey is not known. The ease with which it can be reared and the availability of food plants year around may make the queen of Spain fritillary at attractive species for display in butterfly houses, and it is a valuable model butterfly for further research in genetics, behavior, migratory habits, and physiology.

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