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## LIFE HISTORY AND BIOLOGY OF *PHYCIODES PHAON* (LEPIDOPTERA: NYMPHALIDAE)

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#### ABSTRACT

The butterfly *Phyciodes phaon* (Edwards), the Phaon crescent, was reared in the laboratory on its host plant, *Phyla nodiflora* (L.) Greene, at 27°C with 16:8 (L:D) photoperiod and fluorescent lighting. Eggs are laid in clusters on the underside of host leaves and hatch in about 5 days. Newly hatched larvae aggregate and feed on the underside of the leaf. Later instars disperse on the host plant and continue to feed on the leaves. Larvae develop through five instars based on head capsule, weight, and size measurements. The duration of each instar and the pupal stage were determined. Adults mate 2-3 days after emergence, and females begin laying eggs after 2 more days. The life cycle from egg to adult requires 23-31 days. The butterfly is easy to rear and mating occurs in laboratory cages under artificial lighting. The butterfly has been reared continuously in the laboratory for about 3 years with no evidence of disease in the colony.

Key Words: *Phyciodes phaon* (Edwards), butterfly, Phaon crescent, Nymphalidae, Lepidoptera, insect-host plant interaction, *Phyla nodiflora* (L.) Greene, Verbenaceae

#### RESUMEN

La mariposa, *Phyciodes phaon* (Edwards), fue criada en el laboratorio en su planta hospedera, *Phyla nodiflora* (L.) Greene, a los 27°C con un fotoperíodo de 16:8 (L:D) e iluminación florescente. Los huevos son puestos en grupos en el envéz de la hoja del hospedero y se eclosionan en aproximadamente 5 dias. Las larvas recien nacidas se agregan y se alimentan en el envez de las hojas. Los estadios tardios se dispersan en la planta hospedera y continuan alimentandose sobre las hojas. Las larvas pasan por cinco estadios basado sobre la cápsula de la cabeza, el peso y las medidas del tamaño. La duración de cada estadio y el estado pupal fue determinada. Los adultos se aparearan 2-3 dias después de la salida, y las hembras empiezan poner huevos 2 dias después. El ciclo de vida desde el huevo hasta el adulto requiere 23-31 dias. La mariposa es fácil criar y el apareamiento ocurre en el laboratorio bajo iluminación artificial. La mariposa ha sida criada continuamente en el laboratorio por alrededor de 3 años sin evidencia de una enfermedad en la colonia.

Species of the butterfly genus *Phyciodes* Huebner (Nymphalidae) are restricted to the Americas, and many of the species are tropical. There are 12 species in the United States that have been divided into three species-groups (Scott 1994). The Phaon crescent, *Phyciodes phaon* (Edwards), occurs in Florida (Opler & Krizek 1984; Minno & Minno 1999) and is distributed from coastal North Carolina throughout the southern parts of the Gulf States to southern Texas and westward to southern California, and sometimes migrates north to Iowa and Nebraska. The Phaon crescent adult is characterized by a strong contrasting orange and black coloring of the forewings and upper side of the hindwings. The undersides of the hindwings are pale with brown markings. The Phaon crescent is distinguished from other *Phyciodes* species by having a creamy yellow band evident across both upperside and underside of the forewing.

The host plant utilized by the Phaon crescent in Florida is *Phyla nodiflora* (L.) Greene (previously described as *Lippia nodiflora* L.) in the Verbenaceae (Riley 1975), and it is known by a number of common names including fog fruit, frog fruit, matchweed, capeweed, creeping Charlie and match heads (Verdcourt 1992). It is a perennial herb with long creeping stems and small white to light yellow flowers with a purple center (Fig. 1). It is widely distributed in the southern United States. It roots readily at the nodes and spreads as a ground cover. Leaves are opposite, wedge shaped, thick, leathery, and finely serrated along the edges but rounded at the tip. The plant prefers moist areas and disturbed habitats such as along roadsides and sidewalks, and the margins of wetlands and rivers. Two other butterflies reported to use *Phyla nodiflora* as a larval host are the common buckeye, Junonia coenia Hübner, and the white peacock, *Anartia jatrophae* Munroe (Minno & Minno 1999). Little is known about the biology of *P. phaon*. The aim in this paper is to describe the life history, biology and immature stages of *P. phaon* feeding on its host plant in the laboratory.



Fig. 1. Phyla nodiflora used as a larval food plant by the Phaon crescent.

#### MATERIALS AND METHODS

During the summer of 1999, P. phaon adults (n = 30) were captured in the vicinity of Gainesville, Florida. Eggs were obtained from these adults by placing them in a screen cage with potted host plants, P. nodiflora. Adults were given access to 10% honey solution or Fruit Punch Gatorade® on small cotton balls. Eggs were removed daily, counted, and kept in a Petri dish on moist filter paper. Larvae were fed freshly cut host-plant material. Larval food was changed every other day by transferring all larvae to new plants. Pupae were harvested daily, and transferred to a new cage with a potted host plant. The colony was maintained under controlled laboratory conditions at 27°C, 16:8 (L:D) h photoperiod. The number of instars was determined from data collected from 10 larvae examined each day. Shed larval head capsules were collected, measured, and preserved in 70% ethyl alcohol. Larvae also were weighed and their length measured daily for the 10 individuals to determine the number of instars. Larvae were weighed individually. Data were analyzed by one way ANOVA with Statpak (Northwest Analytical, Inc., Portland, OR), and

when the F value was significant, means were separated by Fisher's Least Significant Difference. Significance was accepted with  $P \le 0.05$ .

#### RESULTS

Description of  $Phyciodes\ phaon\ Edwards\ Immature$  and Adult Stages

Eggs

Females laid eggs in clusters on the undersurface of host leaves (Fig. 2A). In the laboratory, as few as 5 and as many as 187 eggs occurred in clusters. Sometimes eggs were stacked on top of each other. The light green eggs were elliptical, about  $0.63 \pm 0.03$  mm in length and  $0.36 \pm 0.01$  mm in diameter (N = 25 eggs) with a flattened base and slight depression at the micropyle. They were sculptured with 18-20 vertical raised ridges (Fig. 2A, B). Development to hatching required 5.1  $\pm$  0.3 days at 27°C, and the color of the egg changed from light green to brownish black at about 4 days as the mandibles and head of the larva became visible through the chorion.

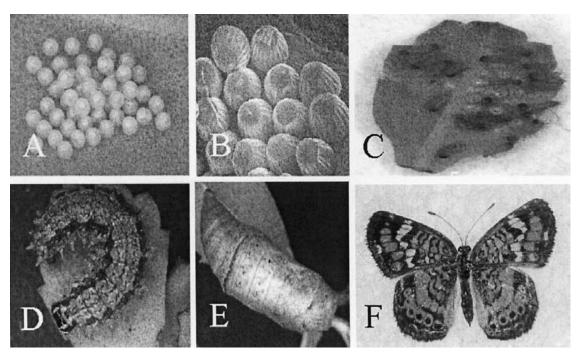


Fig. 2. A composite photo of some developmental stages of the Phaon crescent *Phyciodes phaon*. A, A cluster of eggs on the underside of the host plant; B, Scanning electron micrograph (SEM) of an egg cluster; C, Newly hatched first instars on the underside of a leaf; D, The fourth instar; E, A *P. phaon* pupa attached by the cremaster to a stem of the host plant; F, An adult butterfly.

#### Larvae

Larvae developed through five instars. Larval weight, length, and head capsule measurements (n = 10) in each instar are shown in Table 1. The first instar was olive green to olive brown, with long setae over the body (Fig. 2C). The head was cream colored with two large brown to black patches. The legs and prolegs were light brown and tarsal segments were black to brown. The anal prolegs were dark brown. Antennae were cream in color, with brown basal area. The labrum was brown, the labial and maxillary palpi were light cream in color, and ocelli were black. The facial suture margins were darkened. Head capsule setae were numerous and oriented anteriorly. Brown and cream spots were randomly distributed on the integument. First instars ate their eggshells and stayed aggregated on the underside of the leaf, typically spinning some silk web on the leaf. Generally, larvae rested on top of the silk web, but sometimes larvae rested and fed beneath part of it. They ate small amounts of the underside of the leaf, creating a small pit, which they continued to enlarge as they fed on internal leaf tissue. The duration of the first instar in the laboratory was  $3.6 \pm 0.8$  days (n = 25).

The second instar was light brown in color with dark subdorsal bands. Each segment contained a row of short, branching small spines. The

head was black with two long cream dorsal stripes extending posterior to the neck. The mouthparts were dark brown. The head capsule setae were more numerous than in the first instar. The integument was textured with brown, dark brown, and cream spots. The longitudinal, dorsal and subdorsal bands were more evident in the second instar than in the first instar. The thoracic legs were light brown or cream in color with the tarsal claws darkened. The spiracles were brown. The duration of the second instar was  $3.8 \pm 0.8 \text{ days}$  (n = 25).

Third instars were similar in appearance to second instars, but cream patches on the head capsule were more evident. Third instars generally rested on the upper side of leaves and fed on the edges. They no longer aggregated, but distributed themselves over the whole plant. They spent  $4.1 \pm 0.8$  days in the third instar (n = 25).

Fourth and fifth instars were similar in appearance to each other and to third instars (Fig. 2D). These last two instars consumed a large quantity of host leaves. The duration of the fourth instar was  $4.3 \pm 0.8$  days (n = 25), and duration of the fifth instar was  $3.9 \pm 0.8$  days (n = 25).

#### Prepupae

Mature larvae attached with the cremaster to a stem, leaf or other support and remained in a cres-

Table 1. Measurements of head capsule, weight, and length of Larval  $Phyciodes\ phaon$  in each instar (mean  $\pm$  SD, N = 10).

Instar	Head capsule measurements (mm)	Weight (mg)	Length (mm)
First	$0.296 \pm 0.008$ a	$5.1 \pm 0.7$ a	2.06 ± 0.08 a
Second	$0.593 \pm 0.008 \text{ b}$	$15.5 \pm 4.9 \text{ b}$	$6.07 \pm 0.12 \text{ b}$
Third	$0.798 \pm 0.004 \text{ c}$	$37 \pm 4.8 \text{ c}$	$12.63 \pm 0.43$ c
Fourth	$1.295 \pm 0.007 d$	$81 \pm 7  d$	$18.95 \pm 0.83 d$
Fifth	$1.90 \pm 0.007$ e	$163 \pm 9 \; e$	$28.3 \pm 0.67 e$
LSD*	0.0063	0.0054	0.0456

\*LSD = Fisher's Least Significant Difference between any two means. The means within a column followed by a different letter are different from each other (P < 0.05) (ANOVA and Fisher's Least Significant Difference tests).

cent shape about 8-10 hours. Then, hanging straight down, they changed within 2-3 minutes into the characteristic pupal shape and appearance.

#### Pupae

Pupae were initially very soft and light tan, speckled with black and white (Fig. 2E). They had darker and paler areas over the wings, and a brown "U-shaped" mark around the front of the head. Some pupae were very dark, almost black, in color, but the cause of this color variation was not explored. The pupal abdomen consisted of 10 segments, with the 10th segment bearing the cremaster by which pupae attached to a support. Pupae measured  $12.2 \pm 0.1$  mm in length,  $5.8 \pm 0.1$  mm in width (measured dorsoventrally in the thoracic region), and weighed an average  $82 \pm 40$  mg (n = 25). The duration of the pupal stage was  $4.6 \pm 0.8$  days.

#### Adults

Males and females were similar in appearance (Fig. 2F). The wingspan was  $30.7 \pm 0.02$  mm in females and  $23.4 \pm 0.01$  mm in males (n = 25). Mating pairs often rested quietly together 4-5 hours. Mated females started laying eggs about 2 days after mating. A single female laid from 200-250 eggs (n = 25). Adults survived in the laboratory about 2 weeks. The duration from egg to adult was 23-31 days at 27°C, 16:8 (L:D) photoperiod in the laboratory.

#### DISCUSSION

Species in the genus *Phyciodes* are believed to be a monophyletic group based upon mitochondrial DNA sequences (Wahlberg & Zimmermann 2000). Most of the species feed as larvae on host plants in the family Asteraceae and Acanthaceae (Scott 1994; Brock & Kaufman 2003). In addition to feeding upon the Asteraceae, *P. picta* also colonizes Convolvulaceae, and larvae of the phaon crescent feed on several species in the genus *Phyla* in Verbenaceae and one species in Acan-

thaceae (Scott 1994; Wahlberg 2001). Larval food plants for several species are still unknown (Brock & Kaufman 2003).

The ranges of the phaon crescent and pearl crescent overlap in northern Florida and parts of the southern United States, but the larval host plants belong to two different plant families, the Verbenaceae and Asteraceae, respectively (Oliver 1982; Emmel & Kenney 1997; Brock & Kaufman 2003). Oliver (1982) succeeded in achieving handpaired matings between adults of the phaon crescent and pearl crescent, and obtained  $F_1$  hybrids from some crosses that would feed upon both P. nodiflora and various asters.

In our study, adult phaon crescents mated readily in small to large laboratory cages, and cage size and lighting seemed not to be critical. Although the host plant is widely available in much of the southern United States, it also can be cultured easily in small pots. Remarkably, during three years of rearing the butterfly we have seen no evidence of disease. These ease-of-rearing characteristics and the availability of the host plant all year in the Gainesville area (and possibly further north in protected places) make the phaon crescent a potentially useful teaching tool in schools and a convenient display butterfly for butterfly houses. Moreover, the Phaon crescent seems to be a valuable model butterfly for further research in genetics, mating behavior, pheromone biology, and physiology.

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