

Biology, Ecology and Diversity

Biology of the immature stages of *Strymon crambusa* (Lycaenidae, Theclinae) on Oxalidaceae



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ABSTRACT

We document the biology and morphology of the egg, caterpillar, and pupa of *Strymon crambusa* (Hewitson, 1874), a Neotropical Eumaeini. In the Cerrado, the caterpillar feeds on the inflorescences and leaves of *Oxalis L. S. crambusa* has four larval instars, all of which are illustrated. The density of caterpillars on plants is higher than that recorded for leaf-feeding caterpillars and other flower-feeding Eumaeini, which suggests that the species is a specialist on Oxalidaceae in the Cerrado.

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Introduction

The Eumaeini Tribe (Lycaenidae, Theclinae) is primarily Neotropical (Robbins, 2004) and is often considered the most diverse tribe of butterflies (Papilionoidea) (Fiedler, 1996; Quental, 2008). *Strymon* Hübner is one of the most species-rich genera of Eumaeini (54 species) (Robbins, 2004). It is widely distributed, with some species restricted to dry areas of Central America and South America (Austin and Johnson, 1997; Brown, 1993; Johnson et al., 1990; Nicolay and Robbins, 2005; Robbins and Nicolay, 2002). Some species are highly polyphagous, and more than 30 plant families are cited as food plants for this genus (Beccaloni et al., 2008; Robbins and Nicolay, 2002; Silva et al., 2011).

There are few published works on the biology of *Strymon* immatures in South America, except those related to bromeliads, especially pests of pineapple (Lacerda et al., 2007; Robbins, 2010; Schmid et al., 2010). *Strymon* caterpillars, as well as several other Eumaeini, have specialized in eating the reproductive structures of plants (Badenes-Pérez et al., 2010; Chew and Robbins, 1984; Daniels et al., 2005; Silva et al., 2011). For the Neotropics in general, though, little information is available on the biology of *Strymon* immatures and their food plants (Vila and Eastwood, 2006; Silva

et al., 2014). In the Distrito Federal (DF) of Brazil, there are 10 recorded species of *Strymon* (Brown and Mielke, 1967; Pinheiro and Emery, 2006; Pinheiro et al., 2008), with local food plant records for five of them (Silva et al., 2011). This paper focuses on the biology of the immature stages of *Strymon crambusa* (Hewitson, 1874).

S. crambusa is a medium sized Eumaeini that is rarely sampled in adult inventories (Austin and Johnson, 1997; Paluch et al., 2011). The distribution of this species includes parts of Argentina, Bolivia and Brazil in the states of Rondônia, Pernambuco, Rio de Janeiro, Minas Gerais and DF (Austin and Johnson, 1997; Johnson et al., 1990; Paluch et al., 2011; Pinheiro and Emery, 2006; Zikán and Zikán, 1968). The only recorded food plant for *S. crambusa* is *Oxalis* sp. (Silva et al., 2011), noted during a survey of Eumaeini caterpillars in inflorescences in the Cerrado of DF. The plant has now been identified as *Oxalis densifolia* Mart. and Zucc.

Subsequently, specific searches have been made for immatures of *S. crambusa* on Oxalidaceae and, recently the species was observed also on *Oxalis cordata* A. Saint-Hilaire. The objective of the present work is to document these findings on the biology and morphology of immature *S. crambusa* in the DF of Brazil.

Material and methods

Study area

The survey was conducted in the Fazenda Água Limpa (FAL) (15°55' S–47°55' W) at 1050–1100 m elevation. The FAL is a 4500 ha

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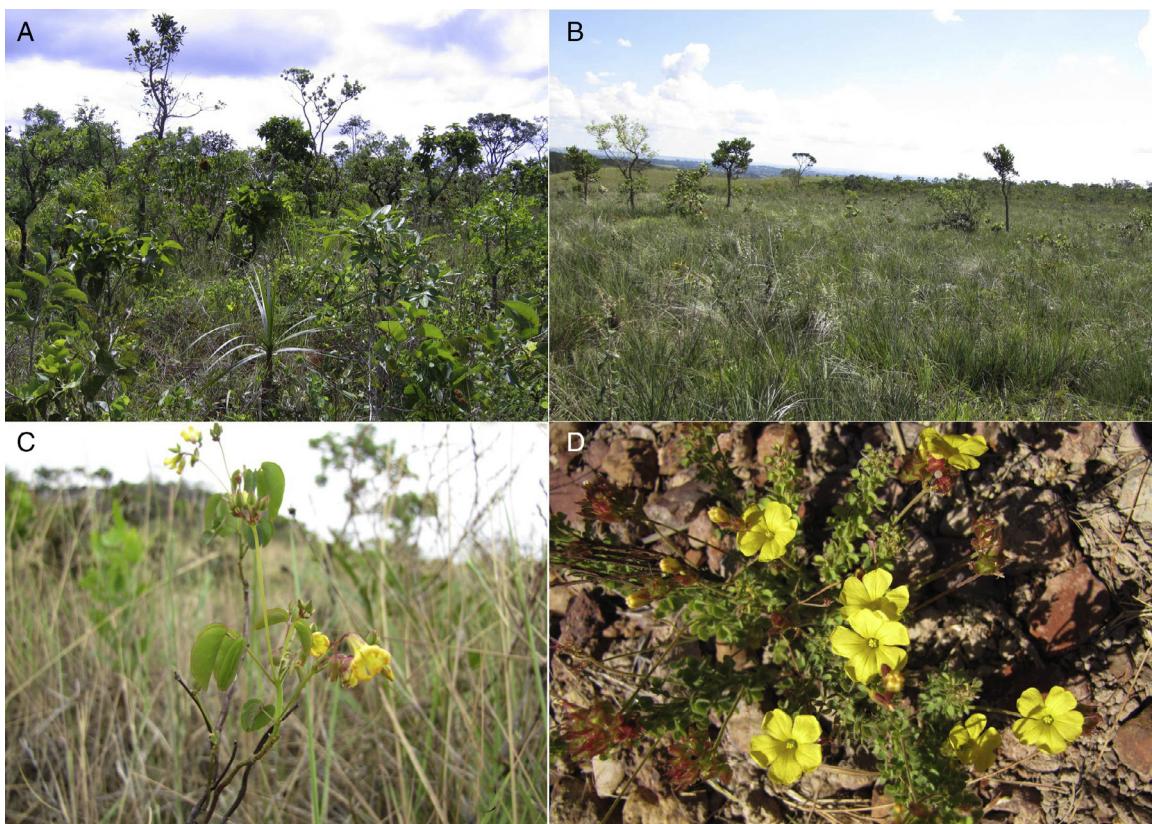


Fig. 1. Host plants and general view of the vegetation of study areas of *Strymon crambusa*. (A) cerrado *sensu stricto*; (B) “campo sujo” (Cerrado grassland); (C) *Oxalis cordata*; (D) *Oxalis densifolia*.

experimental farm with conserved and protected areas of Cerrado vegetation. It belongs to the Universidade de Brasília (UnB), being a part of the core of the Environmental Protection Area of Gama e Cabeça de Veado, DF, Brazil. The FAL vegetation has several Cerrado biome physiognomies, from open areas, such as grasslands, to gallery forests (Ratter, 1980; Munhoz and Felfili, 2005). Plants were searched in random areas of cerrado *sensu stricto* and “campo sujo” (Fig. 1A and B). “Campo sujo” is dominated by herbaceous plants, but may also contain shrubs and semi-shrubs, and cerrado *sensu stricto* is woody savannah with 15–30% tree cover (Munhoz and Felfili, 2006; Oliveira-Filho and Ratter, 2002). The region has defined dry (May–September) and wet (October–April) seasons, with a mean annual precipitation of 1417 mm, and a mean annual temperature of 22 °C (RECOR, 2014).

Food plants

There are approximately 185 recorded species of *Oxalis* L. (Oxalidaceae) in the Neotropics (Fiaschi, 2010). In the Cerrado of DF, this genus is represented by at least 10 native species, popularly known as “trevo” or “azedinha”. *Oxalis* is well known for the presence of oxalic acid in its tissues (Cavalcanti and Ramos, 2001; Reis and Alvim, 2013). Two representative *Oxalis* species in the study areas, *O. cordata* (Fig. 1C) and *O. densifolia* (Fig. 1D), were inspected using similar methodology. These herbaceous shrub species can reach 50 cm tall, allowing the inspection of the plant as a whole. They possess nectaries on their leaves and yellow flowers. Without their flowers, it is not easy to see them in the vegetation. Seasonality of these plants is not well known. Flowering plants have been seen throughout the year, but *O. densifolia* blooms more frequently between October and January (Proença et al., 2006). *O. cordata* loses its leaves at the dry season peak (July and August). The subterranean root system of these *Oxalis* species allows them to re-grow

and flower a few weeks after a fire in the Cerrado (Conceição and Giulietti, 1998; Munhoz and Felfili, 2006).

Collection and rearing

We searched for *S. crambusa* eggs and caterpillars from January to September 2012, and from August 2013 to July 2014. No plant was surveyed more than once. All eggs and caterpillars encountered in the field were collected, transferred to the laboratory in plastic bags with parts of the food plant. They were reared in individual plastic pots without temperature or humidity control. Caterpillars were supplied with the food plant *ad libitum*, and preferences for leaves or inflorescences were observed (following Silva et al., 2014). Head capsules were preserved and measured for each molt. Dead caterpillars and emerged parasitoids in the laboratory were fixed in Kahle solution and then preserved in 70% ethanol. We consulted Downey and Allyn (1984) and Stehr (1987) for the terminology of general morphology of immature stages. Photographs were taken with a Canon® PowerShot SX20 IS digital camera; measurements and general aspects of morphology were analyzed using a Leica® S8APO stereomicroscope with an attached micrometric scale. Measurements are presented as mean and standard deviation when possible. Immature and adult voucher specimens were deposited in the Coleção Entomológica do Departamento de Zoologia, UnB.

Results

We inspected 222 plants of *O. densifolia* and *O. cordata*. We found no immatures of *S. crambusa* on 67 (30.2%) plants without flowers. However, we found nine eggs and 13 caterpillars of *S. crambusa* (Table 1) on 21 (13.5%) of the 155 plants with flowers. These eggs and caterpillars were collected January–March, May,

Table 1

Number of flowering plants examined, plants with egg/caterpillars and number of eggs and caterpillars (no.) and percentage (%) of *Strymon crambusa* collected in two *Oxalis* species (*O. cordata* and *O. densifolia*) in areas of cerrado sensu stricto and "campo sujo" in the Fazenda Água Limpa, DF, Brazil, between 2012 and 2014.

	<i>O. cordata</i>		<i>O. densifolia</i>		Total	
	No.	%	No.	%	No.	%
Examined plants	107	69.0	48	31.0	155	100
Eggs	5	55.6	4	44.4	9	100
Caterpillars	9	69.2	4	30.8	13	100
Plants with egg/caterpillars	14	63.6	7	36.4	21	13.5

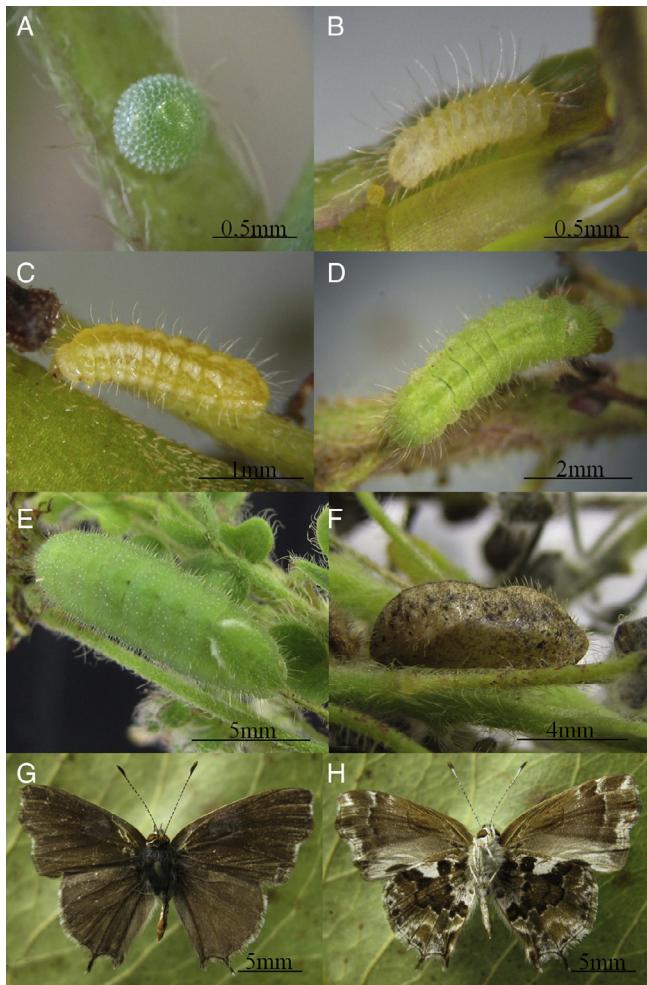


Fig. 2. *Strymon crambusa*, immatures and adult reared under laboratory conditions on *Oxalis*: (A) egg on the peduncle of inflorescences *O. cordata*; (B) first instar, (C) second instar, (D) third instar and (E) fourth instar on *O. densifolia*; (F) pupa; (G) adult male, dorsal view and (H) ventral view.

and August–October. Two eggs failed to hatch, four caterpillars died in different instars (L1–L3), one caterpillar was parasitized, one pupa did not reach adulthood, and 14 were reared to the adult stage (Fig. 2G and H).

Eggs (Fig. 2A) were laid singly on floral buds, on the peduncle of inflorescences and on the leaves. The caterpillars were solitary. Only two eggs were recorded on the same plant of *O. densifolia*, and *S. crambusa* caterpillars co-occurred twice with caterpillars of *Hemiargus hanno* (Stoll) (Lycaenidae, Polyommatusinae). The behavior and coloration of caterpillars were similar on each species of *Oxalis*. First instars feed on flowers, flower buds, and also on young fruits. The second to last instars also feed on leaves. While feeding, the larva holds its body straight or in a slight "s-curve" on

the substrate, moving only its retractable head. In the field, symbiotic interactions with ants were not observed. The larvae became green at the end of the second instar and appeared to be cryptic. In the laboratory, pupation occurred on the plant or on the base of the rearing pot attached by a silk girdle. *S. crambusa* has four instars (Fig. 2). Development time from caterpillar to adult was approximately 30 days. One caterpillar found on *O. densifolia* was parasitized by a species of *Conura* Spinola (Hymenoptera: Chalcididae) that emerged through a hole in the pupal thorax 20 days after pupation.

Immature stages

Egg (Fig. 2A): On *O. cordata* ($n=1$) and on *O. densifolia* ($n=1$). Light green, changing to yellowish before hatching; rounded in dorsal view. Micropylar area slightly depressed and base flattened; exochorion sculptured with prominences at rib intersections. Diameter: 0.50–0.53 mm. Height: 0.27–0.30 mm. Hatching in 4–5 days.

First instar (Fig. 2B): On *O. cordata* ($n=1$) and on *O. densifolia* ($n=1$). Head yellowish, hypognathous projected with the ability to retract it under the thorax. Body onisciform; yellow-whitish prothoracic shield, body and setae. Length of body: 0.98–1.2 mm. Head capsule width: 0.20–0.22 mm. Duration: 5 days.

Second instar (Fig. 2C): On *O. cordata* ($n=1$). Yellowish head and prothoracic shield. Yellow body; two subdorsal and lateral whitish strips; yellowish setae. Length of body: 2.2 mm. Head capsule width: 0.42 mm. Duration: 4 days.

Third instar (Fig. 2D): On *O. densifolia* ($n=1$). Light brown head, greenish body with two cream subdorsal lines; white prothoracic shield; light oblique white lines start to appear, mainly, on the sixth abdominal segment (A6). A scar on the dorsal nectary organ (DNO) present on A7; pore cupola organs (PCOs) observed under a stereomicroscope. Length of body: 4.1 mm. Head capsule width: 0.74 mm. Duration: 3 days.

Fourth instar (Fig. 2E): On *O. cordata* ($n=3$) and on *O. densifolia* ($n=2$). Light brown head. Greenish body with yellowish setae; two oblique white lines on A6–A7, more conspicuous than in L3; light brown spiracles. DNO in A7; PCOs nearby to DNO and spread around the body. In the end of the instar, the caterpillars (prepupae) acquire a uniform tonality (greenish or pinkish). Length of body: 7–11 mm (mean = 8.6 mm ± 1.35). Head capsule width: 1.0–1.1 mm. Duration: 5–6 days (mean = 5.4 days ± 0.54, $n=5$).

Pupa (Fig. 2F): On *O. cordata* ($n=3$) and on *O. densifolia* ($n=2$). Light brown tegument with some irregular black spots; black stripe on the dorsal midline of the abdomen. Body covered with yellowish setae. Pupa is ventrally attached to the substrate with a silken girdle between A1 and A2. Length: 6.27–8.69 mm (mean = 4.8 mm ± 0.88). Duration: 8–12 days (mean = 10 days ± 1.41, $n=5$).

Discussion

Caterpillars of *S. crambusa* resemble other *Strymon* species (Wagner, 2005; Silva et al., 2014). However, the two white oblique lines on the sixth and seventh segments occur more frequently in *S. crambusa* than in *Strymon mulucha* (Hewitson) (N.A.P. Silva, personal observation), another common *Strymon* species in the search area. The number of instars and the development time is similar to other Eumaeini in the Cerrado (Kaminski and Freitas, 2010; Monteiro, 1991). Parasitism by *Conura* is known in Eumaeini species and in general the adult wasp can to emerge within 30 days after the pupation of its host (Silva et al., 2014).

Despite the large number of plant families that are reported to be used by *Strymon*, and of the fact that *Oxalis* species are widely distributed in the Cerrado biome and fields of Brazil (Cavalcanti

and Ramos, 2001; Conceição and Giulietti, 1998; Medeiros, 2011; Proença et al., 2006), Oxalidaceae is unrecorded as a food for any species of Eumaeini. A detritivore was shown to be able to eat leaves of *Oxalis* and to complete development in the lab (Johnson, 1985), but it is unclear whether this plant is used in nature. The polyphagy is common among the flower-feeding Eumaeini in the Cerrado (Kaminski et al., 2012; Silva et al., 2011). However the oligophagy, or the use of a narrow set of phylogenetically closely related plant species, has been more widespread than previously thought in the group (Beccaloni et al., 2008; Fiedler, 1996; Kaminski and Freitas, 2010; Kaminski et al., 2013; Monteiro, 1991; Robbins and Aiello, 1982; Silva et al., 2011, 2014). Ecologically, *S. crambusa* seems to be specialized on Oxalidaceae while *S. mulucha* is recorded on more than seven food plant families (see Silva et al., 2011).

In this study, the interaction between *S. crambusa* caterpillars and ants was not recorded despite the presence of myrmecophilous organs, such as the DNO and the PCOs. We believe that the some interaction between *S. crambusa* and ants may occur because the plants are visited by ants, which are attracted by extrafloral nectaries. Moreover, on *O. densifolia* in FAL, it is not rare to witness *H. hanno* caterpillars (facultative myrmecophilous; see Duarte et al., 2001) interacting with species of genus *Crematogaster* Lund (Formicidae, Myrmicinae) (unpublished data). The presence of the ants on the plant seems to indicate a protected area against natural enemies for the butterflies with myrmecophilous caterpillars. Studies have shown that the diet selection may be ant-dependent; the females prefer to lay eggs on plants that contain ants or that contain ant-tended treehoppers (Atsatt, 1981; Bächtold et al., 2014; Kaminski et al., 2010; Pierce and Elgar, 1985; Wagner and Kurina, 1997).

Another important factor in the female's choice of food plant is the presence/absence of inflorescences. The absence of *S. crambusa* caterpillars on plants without inflorescences in the field indicates that females use visual cues for oviposition (Chew and Robbins, 1984; Rodrigues et al., 2010). For flower-feeding caterpillars, phenological synchrony with the flowering food plant significantly reflects the density of eggs and larval survival (Rodriguez et al., 1994). The current results suggest that *S. crambusa* has a strong local connection with flowering Oxalidaceae.

The role of the secondary plant compounds in lycaenid food plants specificity has yet to be fully explored. Some species of *Oxalis*, for example, are toxic due to the presence of oxalic acid. Moreover, alkaloids, flavonoids and tannins have also been detected in these plants (Violante et al., 2009). The flavonoids, when present in the diet of caterpillars, can be sequestered for multiple functions, including resembling the host plant by changing wing color (Burghardt et al., 2001; Mizokami et al., 2008; Monteiro, 1991). The presence of this compound was evaluated for eggs, caterpillars and the pupae of *Pseudozizeeria maha* (Kollar, 1844), a monophagous Polyommata from an Indian subcontinent, on the leaves of *Oxalis corniculata* L., and the authors suspect that the species also uses the flavonoids as a chemical defense compound (Mizokami et al., 2008; Mizokami and Yoshitama, 2009).

We found no *S. crambusa* caterpillars during surveys of more than 150 plant species (other than *Oxalis*) belonging to 35 families during 10 years of research in the Cerrado of DF (Diniz and Morais, 2002; Silva et al., 2011). Interestingly, the frequency of *S. crambusa* on *Oxalis* plants with flowers (13.5%) is greater than that recorded for leaf-feeding caterpillars in the Restinga (see Monteiro et al., 2007) and in the Cerrado (see Andrade et al., 1995; Morais et al., 1996, 2007; Diniz et al., 2011; Braga et al., 2014). Silva et al. (2011, Table 1) also recorded a lower frequency of Lycaenidae in the extensive survey of inflorescences of different plants. On the other hand, it is known that different species of Lepidoptera can follow the temporal pattern of the host plant (Rodriguez et al., 1994; Muniz et al., 2012), which may not have coincided with collection periods. For example, Vargas (2014) noted a large difference in

the abundance of flower-feeding lycaenids between two successive summers.

The results in this paper are significant because the preference for oviposition sites and larval feeding may represent different defense strategies and are key aspects for understanding the trophic relationships and evolution of Lepidoptera (Duarte and Robbins, 2010; Kaminski et al., 2012). Surveys of caterpillars that feed on inflorescences of plants in the Cerrado of FAL over the last 10 years have revealed a rich and little-known fauna (Diniz and Morais, 2002; Morais et al., 2009; Silva et al., 2011). Future studies in different areas, especially burned areas where the herbaceous species of the Cerrado have an intense flowering a few days or weeks after a fire (Munhoz and Felfili, 2006), can contribute to a better understanding of the ecological requirements and population dynamics of *S. crambusa*.

Conflicts of interest

The authors declare no conflicts of interest.

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