Effectiveness of the aposematic *Eumaeus childrenae* caterpillars against invertebrate predators under field conditions

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Abstract

Effectiveness of the aposematic Eumaeus childrenae *caterpillars against invertebrate predators under field conditions*. *Eumaeus childrenae* (Lepidoptera, Lycaenidae) caterpillars are specialist herbivores that feed on *Dioon holmgrenii* (Cycadacea). They are a well–documented case of chemical protection by sequestering cycasin and related compounds from their host. In this study we evaluated the effectiveness of aposematic defenses against chemical and visual invertebrate predators in wild populations of *E. childrenae* reared on *D. holmgrenii*. The results from field experiments indicated that the estimated survival and the intrinsic rate of increase in cohorts with predator exclusion were twice those in cohorts without predator exclusion. The visually oriented predators observed were *Mischocyttarus* wasps and assassin bugs, and the chemically oriented predators were *Wasmannia*, *Crematogaster* and *Ectatomma* ants. Other mortality factors observed were egg cannibalism, nuclear polyhedrosis virus and, reported for the first time, larval parasitism by fly larvae and a fungus.

Key words: Cycasin, Chemical defense, Chemical ecology, Tritrophic interaction, Demography, Cycads

Resumen

Eficacia de las orugas aposemáticas de Eumaeus childrenae *contra depredadores invertebrados en el medio natural*. Las orugas de *Eumaeus childrenae* (Lepidoptera, Lycaenidae) son herbívoros especialistas que se alimentan de *Dioon holmgrenii* (Cycadacea). Constituyen un caso bien documentado de protección química mediante el secuestro de cicasina y de compuestos relacionados de su hospedante. En este estudio evaluamos la eficacia del aposematismo contra depredadores invertebrados con mecanismos químicos y visuales de caza en una población silvestre de *E. childrenae* criada en *D. holmgrenii*. Los resultados de los experimentos de campo indican que la supervivencia estimada y la tasa de cambio intrínseca fueron el doble en las cohortes con exclusión de depredadores que en aquellas donde no se excluyó a los depredadores. Los depredadores con mecanismos de caza visual observados fueron avispas del género *Mischocyttarus* sp. y chinches, mientras que los depredadores con mecanismos químicos de caza fueron varias especies de hormigas de los géneros *Wasmannia*, *Crematogaster* y *Ectatomma*. Otros factores de mortalidad observados fueron el canibalismo de huevos, el virus de la poliedrosis nuclear y el parasitismo de las larvas por larvas de moscas y un hongo, que se observan por primera vez.

Palabras clave: Cicasina, Defensa química, Ecología química, Interacción tritrófica, Demografía, Cícadas

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Introduction

Chemically defended animals often deter predators by stimulating their visual and chemical sensory channels (Rojas et al., 2018). The aposematic coloration of prey is a defense mechanism against visual predators (Dell'Aglio et al., 2016). Aposematic prey are under positive frequency–dependent selection, whereby the efficiency of a defense signal increases in relation to local abundance and background (Seymoure et al., 2018). Chemically protected or unpalatable prey have chem2 s ical compounds with a noxious taste or odor that deters predators (Brower, 1984). Once predators have tasted their toxins, they will usually avoid them (Leimar et al., 1986). Plant–derived chemical defense has been documented in many lepidopteran species (Opitz and Müller, 2009), such as the *Eumaeus* species. This species sequesters cycasin and related compounds from its cycad plant hosts (Rothschild et al., 1986; Bowers and Larin, 1989; Nash et al., 1992).

The butterfly *Eumaeus childrenae* Gray is a specialist herbivore that feeds on *Dioon holmgrenii* De Luca, Sabato and Vázquez Torres (1981) an endemic cycad species of the physiographic province Sierra Madre del Sur and the Pacific Coastal Plain of the state of Oaxaca, Mexico (Cervantes–Zamora et al., 1990). The cycad *D. holmgrenii* is considered threatened and endangered due to its low genetic diversity (González–Astorga et al., 2008) and disturbance of its habitat (Velasco–García et al., 2016). Previous research confirms that this species of the genus *Eumaeus* sequesters and accumulates cycacin when feeding on cycad leaves and strobili (Rothschild et al., 1986; Bowers and Larin, 1989). Observational and feeding experiments suggest that cycasin in eggs and larvae produces a deterrent effect against ants (Bowers and Larin, 1989; Castillo–Guevara and Rico–Gray, 2002) and makes *E. atala* adults unpalatable to birds (Bowers and Farley, 1990). It thus provides chemical protection against predators throughout all stages of the *E. atala* life cycle (Rothschild et al., 1986). This chemical protection is signaled to its predators through the red coloration of the larvae (Schneider et al., 2002) that is magnified by their gregarious behavior (Bowers and Farley, 1990). While these laboratory studies demonstrate that the sequestration of cycasin and related compounds are potentially important determinants of predation rates, no field experiments have yet been performed to test the deterrent effect of cycasin and aposematism against other predators such as mites and spiders (Beltrán–Valdez and Torres–Hernández, 1995) or against unreported predators, parasites and diseases in wild populations.

We investigated the effectiveness of specific defenses against multiple species (Camara, 1997) and report the results of field experiments that measured the efficacy of aposematic defenses against chemical and visual predators in wild populations of *E. childrenae* reared on its natural host plant, *D. holmgrenii*. We used the survival rate from oviposition until adult emergence and the intrinsic rate of reproduction to measure deterrent efficiency against all invertebrate predators and parasites in the life cycle of *E. childrenae* in two conditions: with and without predator exclusion. An additional aim was to investigate the possible presence of new predators and parasites in the Neotropical region.

Material and methods

Study area

The demographic parameters of *E. childrenae* were determined in the locality of *D. holmgrenii* located at kilometer 244 of the Oaxaca to Puerto Escondido highway, at 16º 1' 47.1'' N and 97º 3' 59.9'' W. It has an area of approximately 4.9 hectares, at 650 m to 850 m a.s.l.. The climate is warm and subhumid with rains in summer, the most humid of the subhumid, with percentage of winter rain less than 5 [$Aw_2(w)$]. The average annual temperature is 26 ºC and the average annual rainfall is 1,500 mm (INEGI et al., 2008). Vegetation corresponds to a transition zone between oak forest and sub–deciduous forest.

Cohorts

From June 2015 to February 2017 we recorded 29 cohorts with 1404 eggs on accessible host plants. Survival rates until adult emergence were estimated in 21 cohorts and 1,215 eggs; eight cohorts that were completely destroyed or that moved to inaccessible plants were excluded. The cohorts were grouped according to two rearing conditions: without predator exclusion (WPE) (14 cohorts with 819 eggs on leaves and 5 cohorts with 283 eggs on strobili), and with predator exclusion (PE) (2 cohorts with 113 eggs on leaves). The PE cohorts were placed in exclusion using a cylindrical cage (1 m long per 0.5 m diameter). The cage was wired and covered with a fine mesh that excluded predators such as ants, wasps, bugs and birds. The caged larvae were fed host cycad leaves cut from the most recent flush of leaves. The pupae of three cohorts that grew on WPE leaves were kept in net screen cages (1.25 × 1.25 × 1.25 m) until adult emergence. In both cages, the sex ratio and the number of oviposited eggs were recorded in order to calculate the fertility table. Each cohort was visited every day to record the time of molting. The behavioral response of the larvae when attacked by the predators was recorded. The behavior of each predator species observed attacking larvae was also recorded, including predation on other prey. Digital photographs were taken to document the predators observed in each stage.

Data analysis

The survival profile was estimated using the Kaplan–Meier nonparametric estimator. A pairwise comparison of survival profiles considering reared conditions was used with a Mantel–Haenszel test. The observed significance level was adjusted for the number of comparisons using the Bonferroni procedure (Lee and Wang, 2003). The fertility table was built using a simple
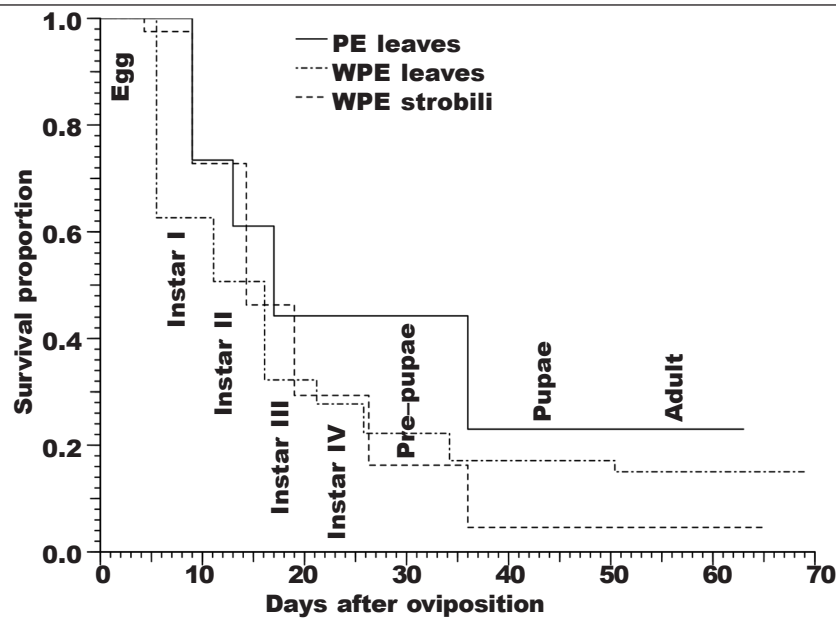
Fig. 1. Survival rate of *Eumaeus childrenae* from egg to adult emergence in leaf and stroboli of *Dioon holmgrenii* under field conditions in the physiographic province of Sierra Sur, Oaxaca, Mexico: PE, with predation exclusion; WPE, without predation exclusion.

Fig. 1. Índice de supervivencia de Eumaeus childrenae *desde el huevo hasta la aparición del adulto en hojas y estróbilos de* Dioon holmgrenii *en el medio natural en la provincia fisiográfica de Sierra Sur, Oaxaca, en México: PE, con* exclusión de depredadores; WPE, sin exclusión de depredadores*.

decrement abridged life table grouped by stages, the number of eggs, and the sex ratio observed in cages. The net reproduction rate (R_0), mean generation time (T_C) and intrinsic rate of increase (r $\approx$ ln $R_0 \cdot T_C^{-1}$) were estimated following Carey (1993).

Results

The survival rate up until to adult emergence differed between cohorts on WPE leaves, WPE strobili, and PE leaves (χ^2 = 16.4, p < 0.000272). The survival rate (0.230 ± 0.039) was highest on PE leaves, followed by WPE leaves (0.15 ± 0.039) and WPE strobili (0.046 ± 0.012). Figure 1 shows the survival profile for each rearing condition. We merged the survival rates on WPE leaves and WPE strobili because they were similar (χ^2 = 0.8, p < 0.374). The WPE survival rate (0.123 ± 0.029) was lower (χ^2 = 12.5, p < 0.001) than that in the PE cohorts. In the PE cohorts, the net reproduction rate was 0.436 individuals per female, with a generation time of 53.2 days; the estimated intrinsic rate of increase was –4.8. In the WPE cohorts, the net reproduction rate was 0.244 individuals per female, with a generation time of 54 days, and the estimated intrinsic rate of increase was –5.4. In both cages, the observed sex ratio was 1:1 (exact binomial test: 0.437, p = 0.261). The average number of oviposited eggs on leaves was 54.6 ± 6.7, and 69.7 ± 6.7 on strobili.

Mortality factors observed on eggs were lack of hatching in complete cohorts of up to 65 eggs, and the sucking of deutoplasm through the micropyle by recruiting and mass attack by ants of the genera *Wasmannia* sp. and *Crematogaster* sp. (Hymenoptera, Formicidae) (fig. 1s–A, 1s–B in supplementary material). The attacks by these two species of ants destroyed complete cohorts of up to 93 eggs during the rainy season (June to September). These ants carry the whole dead caterpillar to their nest (fig. 1s–C in supplementary material). They were not observed attacking live caterpillars. Complete destruction of cohorts also occurred as a result of egg cannibalism by first instar larval stages (fig. 3s in supplementary material). Also, a beetle of the genus *Dasydactylus* sp. (Coleoptera, Languriidae) occasionally ate the eggs, biting and killing larvae and pupae (fig. 2s in supplementary material). In the first and second instar larval stages we observed greater natural mortality in both conditions, that is, both with and without predator exclusion cages. One possible explanation for this observation is the antiherbivory defense of cycasyn and related compounds, but this antiherbivory effect has not been documented previously. First and second instar larvae were predated by solitary ants of the genus *Ectatomma tuberculatum* (Hymenoptera, Formicidae) (fig. 1s–D in supplementary material). At the beginning of the dry season (October), assassin bug nymphs sucked the haemolymph of third and

fourth instar larvae, prepupae and pupae, leaving behind dry exoskeletons (fig. 5s in supplementary material). A species of wasp *Mischocyttarus* sp. and *Mischocyttarus mexicanus* ssp. *mexicanus* (Hymenoptera, Vespidae) were also observed killing third and fourth instar larvae and prepupae by biting caterpillars, which were then chewed and swallowed (fig. 4s in supplementary material). These wasps killed up to 27 % of one cohort. This wasp partitioned the prey into chunks for manageable mastication and consumption. They did not sting the caterpillars. Mortality of third and fourth instar larvae was observed during the rainy season, showing typical symptoms of polyhedrosis virus (fig. 6s–A in supplementary material). The dead larvae were transported by ants of the genus *Crematogaster* sp. to their nest. Prepupal mortality was observed at the beginning of the dry season, showing typical symptoms of polyhedrosis virus, which caused 40 % mortality in one cohort (fig. 6s–B in supplementary material). Parasitism of pupae by fly larvae was also observed during the rainy season (fig. 8A–8B in supplementary material). A tachinid adult fly was observed visiting the pupae, but parasitized pupae were not caged in order to obtain adult parasitoids. Parasitism by a fungus in prepupae and pupae was observed during the rainy season (fig. 7s–C, 7s–8D in supplementary material). The parasitized pupae were not collected to identify the fungus. Furthermore, adult individuals were observed trapped in spider webs.

Discussion

The bright red colouring of *Eumaeus* species, which is magnified by the gregarious habits of the larvae, is produced by cycasin and protects against predators (Bowers and Larin, 1989; Nash et al., 1992; Schneider et al., 2002). However, the relevance of the deterrent effect of chemical protection against predators on the survival and reproduction rate of *Eumaeus* has not yet been determined.

The cluster of white eggs, which are quite visible on the leaves and strobili of *D. holmgrenii*, are similar to those reported on *E. atala* reared in *Zamia integrifolia* lf, a case of egg protection by aposematism (Rothschild et al., 1986; Schneider et al., 2002). This hypothesis could be correct in the case of visual predators, because only chemically–oriented predators such as ants were observed consuming and transporting eggs. Even though the deterrent effect of cycasin against ants has been verified in laboratory assays (Bowers and Larin, 1989; Castillo–Guevara and Rico–Gray, 2002), the continuous foraging activity of individuals of *Wasmannia* sp. and *Crematogaster* sp. means that when the first individuals stop feeding due to the deterrent effect, other individuals arrive and continue the foraging until the entire cohort is destroyed. It has been documented that ants use chemical detection mechanisms to hunt (Greeney et al., 2012). Thus, cycasin content in eggs would be expected to have a deterrent effect. However, ants have been found to be the main predators of *Eumaeus* species (Castillo–Guevara and Rico–Gray, 2002; Smith, 2002), not only consuming deutoplasm, but also transporting *E. childrenae* eggs and larvae (both dead and alive) to their nests. The social ant predators were not observed attacking live caterpillars, but transporting dead caterpillar to their nest, suggesting that cycasin was not an important defense against social ant predators. One possibility is that the larvae size deterrent social ants from attacking. The social ants have been observed attacking smaller live larvae (fig. 1s–E in supplementary material), such as *Rhopalotria* sp. larvae, which also contain cycasin inside intact idioblast cells in their gut (Vovides, 1991). Conversily, the aposematism and cycasin do not deter freely foraging ants from attacking first and second instar larvae that are gregarious. *E. tuberculatum* ants are common pretators on *Dioon* plants and male strobili. They capture live larvae and adult *Rhopalotria* sp., *Pharaxonotha* sp. and bees that using strobili (fig. 1s–F in supplementary material). Furthermore, in another species cosidered protedted by aposematism, it has been documented that the larvae have others morphological defenses that deter ant (de la Fuente et al., 1994/1995; Dyer, 1997; Massuda and Trigo, 2009).

Like other plant–feeding Lepidoptera (Lefèvre et al., 2012), newly hatched caterpillars often feed initially on their chorion, but when there are two or more clutches of eggs, the caterpillars that hatched first fed on the unhatched eggs of other clutches (cannibalism), devouring a complete cohort of 113 eggs. This behavior has been reported in other species of Lepidoptera (Barros–Bellanda and Zucoloto, 2001).

The bright red colouring and its magnification by agglomeration constitute a defense mechanism against predators such as birds and mantids (Greeney et al., 2012), wasps and assassin bugs (Johnson, 1983; Dyer, 1997) that rely on visual predation mechanisms (Ruxton and Sherratt, 2006). In the present field study, we did not observe bugs and wasps attacking the gregarious first and second instar larvae. Both wasps and bugs were observed attacking the third and fourth instar larvae, which are larger and live solitarily. However, upon contact with wasps or bugs, caterpillars showed active responses, including escape and lifting their posterior segments. In contrast, *Junonia coenia* Hübner, another caterpillar considered protected by its aposematism, shows no behavioral defenses when attacked by the wasp *Polistes fuscatus* Fabricius (Stamp, 1992). In another study, wasps (*Polistes instabilis* Saussure) were deterred by brightly colored prey (Dyer, 1997). Both previously cited studies support general theories about aposematism. However, our field observations indicate that both species of *Mischocyttarus* wasp are not dettered by aposematism because the feed on larvae and pre–prepupae during moulting process, when they are aggregated and immobile (fig. 4s–C.1, 4s–C.2 in supplementary material). This observation has not been reported previously and suggests that cycasin and aposematic colouring are not useful defense against these wasps. Even

higher predation was reported in another aposematic caterpillar, *Hemileuca lucina* (Saturnidae), with the wasp *Polistes* sp., which killed up to 99 % of one cohort (Stamp and Bowers, 1988).

The only larval disease reported is the polyhedrosis virus (Koi and Daniels, 2015). No other diseases or parasites have been reported to affect the *Eumaeus* species. Pupae were parasitized by fly larvae during the rainy season. Flies are generally considered generalist parasitoids that are little affected by plant allelochemicals sequestered by their larval host (Mallampalli et al., 1996). Fungi and the polyhedrosis virus were also observed parasitizing pupae during the rainy season. There are no previous experiments about unpalatable effects of deterrent contents in the pupae against predators, parasites and diseases. A possible explanation for the parasitism observed in the present study is the increased susceptibility of pupae due to the decrease in their cycasin content. This decrease amounted to 75 % compared to the content of cycasin in fourth instar larvae (Schneider et al., 2002).

The survival rate until adult emergence in the WPE cohorts was half that observed in the PE cohorts. In addition, the estimated intrinsic rate of increase was greater in PE cohorts. Thus, we suggest that chemical protection was insufficient to protect *E. childrenae* and that the observed mortality factors are important top–down forces against this herbivore in wild conditions. This could also be true for the other species of the genus *Eumaeus*, since the average mortality rate observed in the present study was similar to the values reported for eggs (31.25 %) and larvae (64.9 %) of *Eumaeus atala* Godart reared on the cycad *Zamia loddigesii* Miq. (Castillo–Guevara and Rico–Gray, 2002), and to *E. atala* survival rate (17.1 %) from egg to pupae when reintroduced to its host *Z. pumila* (Smith, 2002).

Although aposematic coloration and chemical defenses are well–known defenses against predators in the *Eumaeus* species, our field data suggest that some invertebrate predators are not deterred by warning coloration or chemical protection. Also, our results suggest that the ecological importance of aposematic coloration and chemical defenses as protective mechanisms are overestimated and other strategies against enemies are undervalued, because caterpillars also show strategies such as behavioral responses against predators. Upon contact with wasps or bugs, caterpillars show behavioral responses against enemies, mainly evasive responses, including escape and lifting their posterior segments. Another defense observed in the field is the hiding position of feeding larvae (e.g. under leaflets and sporophylls) to avoid encounters with predators.

Acknowledgements

References

Barros–Bellanda, H. C. H., Zucoloto, F. S., 2001. Inflence of chorion ingestion on the performance of *Ascia monuste* and its association with cannibalism. *Ecological Entomology*, 26: 557–561.

Beltrán–Valdez, J. M., Torres–Hernández, L., 1995. Aspectos cualitativos de la herbivoría de *Eumaeus debora* Hubner. (Lepidoptera: Lycaenidae) sobre *Ceratozamia mexicana* Bröng. (Cycadales: Zamiaceae). *La ciencia y el Hombre*, 19: 91–104.

Bowers, M. D., Farley, S., 1990. The behaviour of grey jays, *Perisoreus canadensis*, towards palatable and unpalatable Lepidoptera. *Animal Behaviour*, 39: 699–705.

Bowers, M. D., Larin, Z., 1989. Acquired chemical defense in the lycaenid butterfly, *Eumaeus atala*. *Journal of Chemical Ecology*, 15: 1133–1146.

Brower, L. P., 1984. Chemical defense in butterflies. In: *The biology of butterflies*: 109–132 (R. I. Vane–Wright, P. R. Ackery, Eds.). Academic Press, London.

Camara, M. D., 1997. Predator responses to sequestered plant toxins in buckeye caterpillars: are tritrophic interactions locally variable? *Journal of Chemical Ecology*, 23: 2093–2106.

Carey, J. R., 1993. *Applied demography for biologists with special emphasis on insects*. Oxford University Press, New York.

Castillo–Guevara, C., Rico–Gray, V., 2002. Is cycasin *Eumaeus minyas* (Lepidoptera: Lycaenidae) a predator deterrent? *Interciencia,* 27: 465–470.

Cervantes–Zamora, Y., Cornejo–Olgín, S. L., Lucero–Márquez, R., Espinoza–Rodríguez, J. M., Miranda–Viquez, E., Pineda–Velázquez, A., 1990. Provincias fisiográficas de México, escala 1:4000000. In: *Clasificación de regiones naturales de México 1. Tomo II, Sección IV, 10.1. Atlas nacional de México (1990–1992)*. Instituto de Geografía, UNAM, México.

de la Fuente, M., Dyer, L. A., Bowers, M. D., 1994/1995. The iridoid glycoside, catalpol, as a deterrent to the predator *Camponotus floridanus* (Formicidae). *Chemoecology*, 5/6: 13–18.

Dyer, L. A., 1997. Effectiveness of caterpillar defenses against three species of invertebrate predators. *Journal of Research on the Lepidoptera*, 34: 48–68.

Dell'Aglio, D. D., Stevens, M., Jiggins, C. D., 2016. Avoidance of an aposematically coloured butterfly by wild birds in a tropical forest. *Ecological Entomology*, 41: 627–632.

González–Astorga, J., Vergara–Silva, F., Vovides, A. P., Nicolalde–Morejón, F., Cabrera–Toledo, D., Pérez–Farrera, M. A., 2008. Diversity and genetic structure of three species of *Dioon* Lindl. (Zamiaceae, Cycadales) from the Pacific seaboard of Mexico. *Biological Journal of the Linnean Society*, 94: 765–776.

Greeney, H. F., Dyer, L. A., Smilanich, A. M., 2012. Feeding by lepidopteran larvae is dangerous: A review of caterpillars' chemical, physiological, morphological, and behavioral defenses against natural enemies. *Invertebrate Survival Journal*, 9: 7–34.

INEGI, CONABIO, INE, 2008. *Ecorregiones terrestres de México*. Escala 1:1000000. México.

Johnson, L. K., 1983. *Apiomerus pictipes*. In: *Costa Rican natural history*: 684–687 (D. H. Janzen, Ed.). University of Chicago Press, Chicago.

Koi, S., Daniels, J., 2015. New and revised life history of the Florida hairstreak *Eumaeus atala* (Lepidoptera: Lycaenidae) with notes on its current conservation status. *Florida Entomologist*, 98: 1134–1147.

Lee, E. T., Wang, J. W., 2003. Statistical methods for survival data analysis. John Wiley & Sons, Inc., New Jersey.

Lefèvre, T., Chiang, A., Kelavkar, M., Li, H., Li, J., Lopez–Fernandez de Castillejo, C., Oliver, L., Potini, Y., Hunter, M. D., de Roode, J. C., 2012. Behavioural resistance against a protozoan parasite in the monarch butterfly. *Journal of Animal Ecology*, 81(1): 70–79.

Leimar, O., Enquist, M., Sillen–Tullberg, B., 1986. Evolutionary stability of aposematic coloration and prey unprofitability: a theoretical analysis. *The American Naturalist,* 128: 469–490.

Mallampalli, N., Barbosa, P., Weinges, K., 1996. Effects of condensed tannins and catalpol on growth and development of *Compsilura concinnata* (Diptera: Tachinidae) reared in gypsy moth (Lepidoptera: Lymantriidae). *Journal of Entomological Science*, 31: 289–300.

Massuda, K. F., Trigo, J. R., 2009. Chemical defence of the warningly coloured caterpillars of *Methona themisto* (Lepidoptera: Nymphalidae: Ithomiinae). *European Journal of Entomology*, 106: 253–259.

Nash, R. J., Bell, A. A., Ackery, P. R., 1992. The protective role of cycasin in cycad–feeding lepidoptera. *Phytochemistry*, 31: 1955–1957.

Opitz, S. E. W., Müller, C., 2009. Plant chemistry and insect sequestration. *Chemoecology*, 19: 117–154.

Rojas, B., Burdfield–Steel, E., De Pasqual, C., Gordon, S., Hernández, L., Mappes, J., Nokelainen, O., Rönkä, K., Lindstedt, C., 2018. Multimodal aposematic signals and their emerging role in mate attraction. *Frontiers in Ecology and Evolution*, 6: 93.

Rothschild, M., Nash, R. J., Arthur, B. E., 1986. Cycasin in the endangered butterfly *Eumaeus atala*. Florida. *Phytochemistry*, 25: 1853–1854.

Ruxton, G. D., Sherratt, T. N., 2006. Aggregation, defence and warning signals: the evolutionary relationship. *Proceedings of the Royal Society B*, 273: 2417–2424.

Schneider, D., Wink, M., Sporer, F., Lounibos, P., 2002. Cycads: their evolution, toxins, herbivores and insect pollinators. *Naturwissenschaften*, 89: 281–294.

Seymoure, B. M., Raymundo, A., McGraw, K. J., Owen–McMillan, W., Rutowski, R. L., 2018. Environment–dependent attack rates of cryptic and aposematic butterflies. *Current Zoology*, 64: 663–669.

Smith, E. M., 2002. The effects of season, host plant protection, and ant predators on the survival of *Eumaeus atala* (Lycaenidae) in re–establishments. *Journal of the Lepidopterists' Socciety*, 56: 272–276.

Stamp, N. E., 1992. Relative susceptibility to predation of two species of caterpillar on plantain. *Oecologia*, 92: 124–129.

Stamp, N. E, Bowers, M. D., 1988. Direct and indirect effects of predatory wasps (*Polistes* sp.: Vespidae) on gregarious caterpillars (*Hemileuca lucina*: Saturniidae). *Oecologia*, 75: 619–624.

Velasco–García, M. V., Valdez–Hernández, J. I., Ramírez–Herrera, C., Hernández–Hernández, M. L., López–Upton, J., López–Mata, L., López–Sánchez, H., 2016. Estructura, heterogeneidad de estadios y patrón de dispersión espacial de *Dioon holmgrenii* (Zamiaceae). *Botanical Sciences*, 94: 75–87.

Vovides, A. P., 1991. Cone ideoblasts of eleven cycad genera: morphology distribution and significance. *Botanical Gazette*, 152: 91–99.
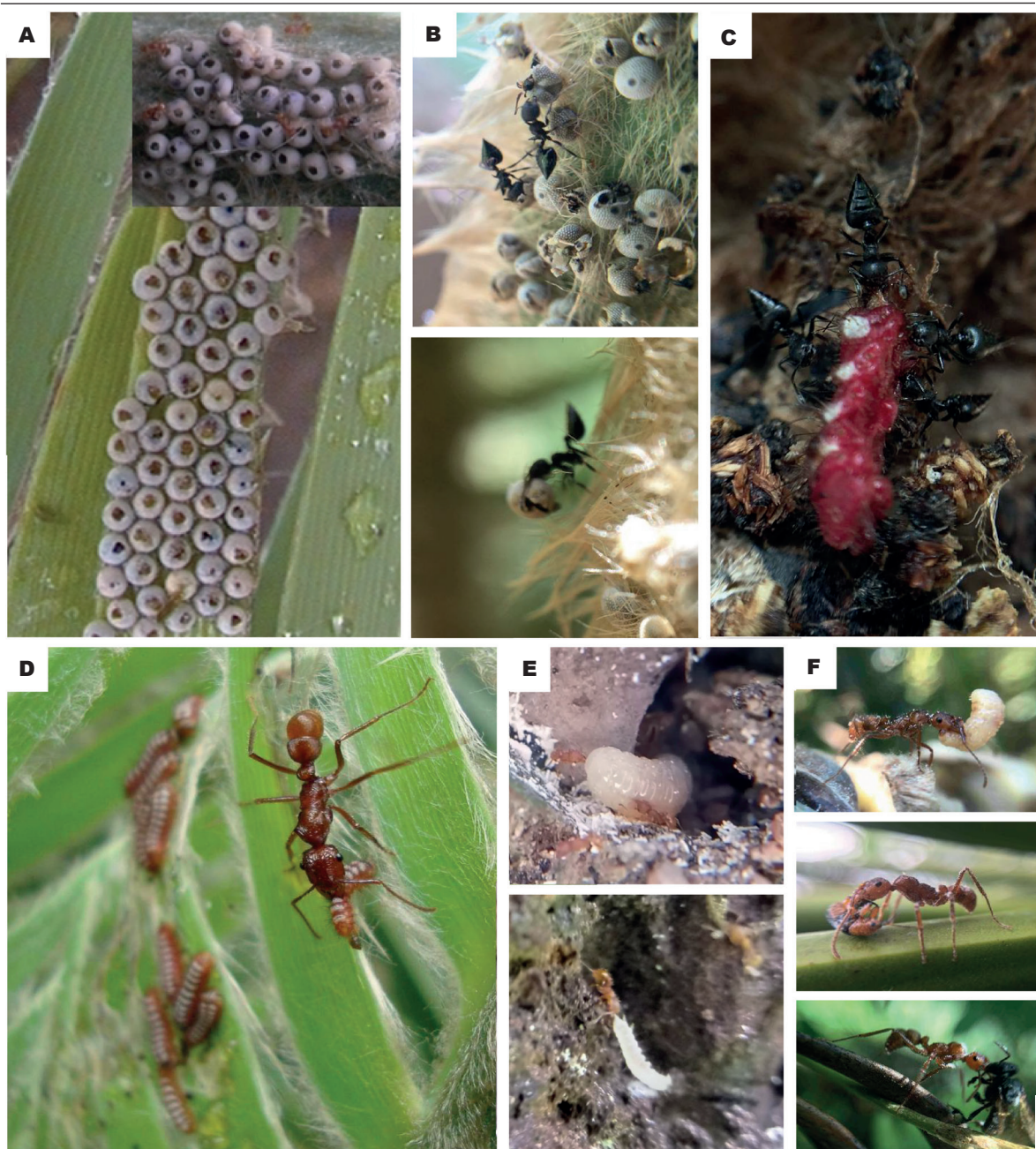
Supplementary material

Fig. 1s. Depredation of egg and caterpillar of *Eumaeus childrenae* by ants: A, the *Wasmannia* sp. ants sucking deutoplasm through the micropyle; B, the *Crematogaster* sp. predating eggs; and C, transport dead caterpillar; D, *Ectatomma tuberculatum* ants transport live caterpillar; E, social ants feed on live larvae of *Rhopalotria* sp. and other unidentified larvae; F, *E. tuberculatum* ants feed on live caterpillar and adult of *Rhopalotria* sp. and bees that visit male strobili.

Fig. 1s. Depredación de los huevos y las orugas de Eumaeus childrenae *por hormigas: A, hormigas del género* Wasmannia *succionando deutoplasma a través del micrópilo; B, hormigas* Crematogaster *sp. depredando huevos; C, transporte de un oruga muerta; D, una hormiga de* Ectatomma tuberculatum *transportando una oruga viva; E, hormigas sociales alimentándose de larvas vivas de* Rhopalotria *sp. y otras larvas sin identificar; F, hormigas de* E. tuberculatum *alimentándose de orugas y adultos vivos de* Rhopalotria *sp. y abejas que visitan los estróbilos macho.*

Fig. 2s. A, depredation of eggs by beetle *Dasydactylus* sp.; B, killing and ate fourth instar caterpillar; C, Ate on exuviae of second instar larval.

Fig. 2s. A, escarabajo Dasydactylus *sp. alimentándose de huevos; B, matando y alimentándose de una oruga de cuarto instar; C, alimentándose de la exuvia de una larva de segundo instar.*

Fig. 3s. Cannibalism of egg by first instar larval.

Fig. 3s. Canibalismo de huevos por larvas de primer instar.

Fig. 4s. Predation of caterpillar by wasp: A, *Mischocyttarus* sp. bitter third instar larval; B, consuming caterpillar of second instar; C.1, *Mischocyttarus mexicanus* ssp. *mexicanus*; and C.2, *Mischocyttarus* sp. biting and killing third instar caterpillar during moulting time.

Fig. 4s. Una avispa alimentándose de una oruga. A, Mischocyttarus *sp. mordiendo una larva de tercer instar; B, consumiendo una oruga de segundo instar; C.1,* Mischocyttarus mexicanus *ssp.* mexicanus*; y C.2,* Mischocyttarus *sp. mordiendo y matando orugas de tercer instar durante el período de muda.*
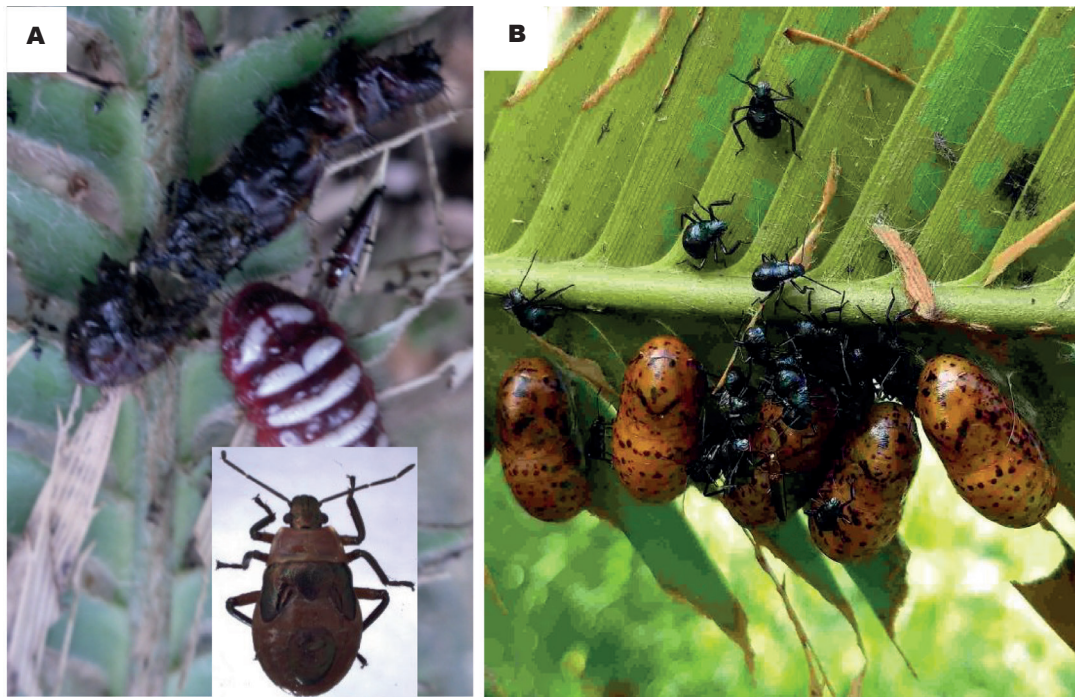
Fig. 5s. Bug nymphs sucking the haemolymph: A, dry exoskeletons of fourth instar caterpillar; B, first instar nymph sucking the haemolymph of pupae.

Fig. 5s. Muerte por succión de la hemolinfa por chinche: A, exoesqueleto vacío de larvas de cuarto instar; B, succión de hemolinfa en pupas por ninfas de primer instar

Fig. 6s. Caterpillar mortality with typical symptoms of polyhedrosis virus: A, fourth instar caterpillar and pre-pupae during rainy season; B, pre–pupae at beginning of the dry season; C, a polyhedral inclusion bodies obtained of dead caterpillar (1,000 x).

Fig. 6s. Muerte de larvas con síntomas típicos del virus de la poliedrosis nuclear: A, larva del cuarto instar en la época lluviosa; B, larvas del cuarto instar y pre pupas en la temporada de secas; C, cuerpos de inclusión poliédricos observados en muestras obtenidas de larvas muertas (1.000 x).
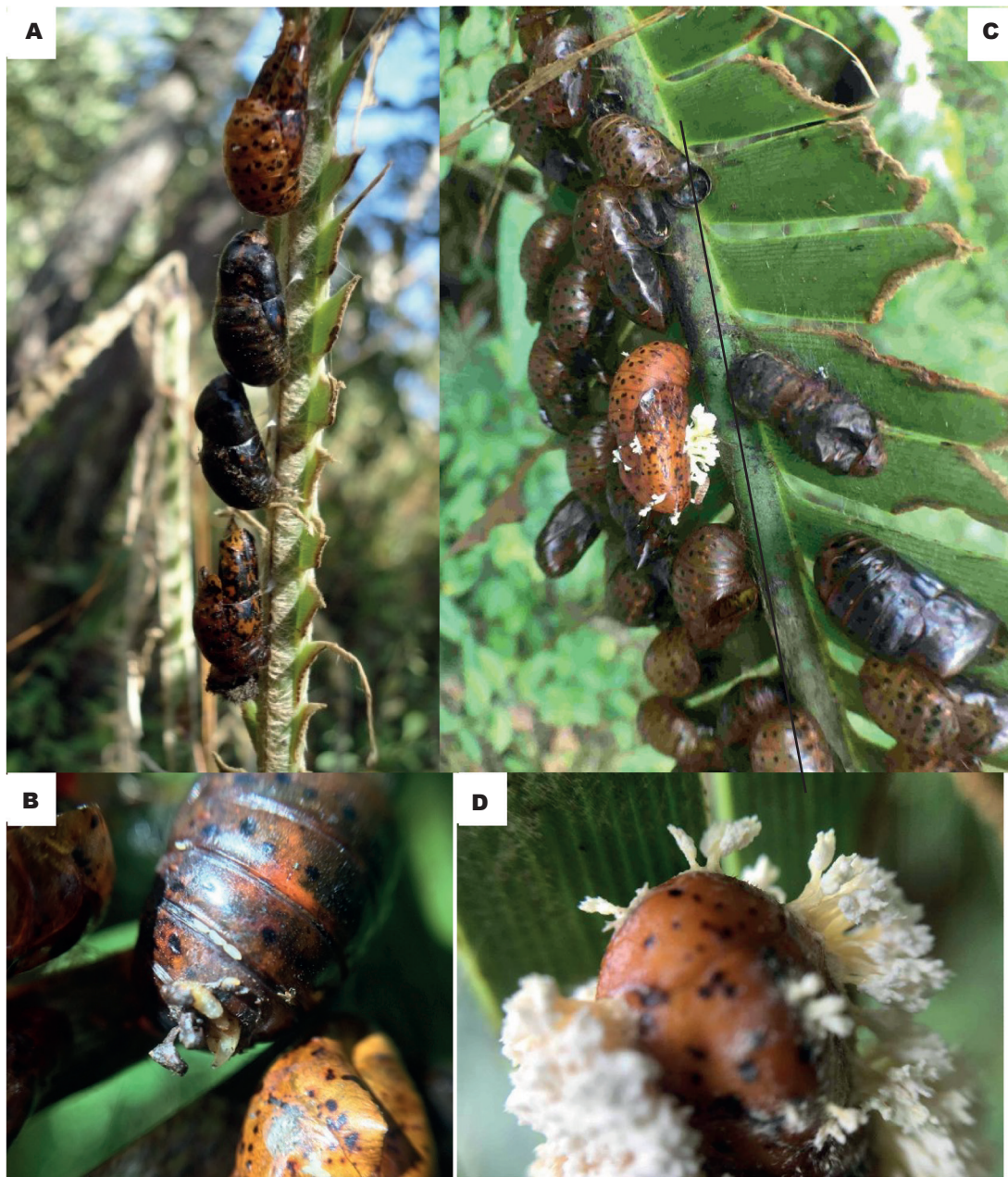
Fig. 7s. Parasitism on pupae during rainy season: A, parasite pupae by fly larvae; B, detail of a fly larvae; C, parasite pupae by a fungus; D, a detailed of fructification structure of fungi.

Fig. 7s. Parasitismo en las pupas durante la temporada de lluvias: A, pupas parásitada por larvas de mosca; B, detalle de una larva de mosca; C, pupa parasitada por un hongo; D, detalle de la fructificación del hongo.