GENERA OF ANTS ASSOCIATED WITH LARVAE OF PLAINS CUPID (*CHILADES PANDAVA*, HORSFIELD, 1829) (INSECTA: LEPIDOPTERA: LYCAENIDAE) INFESTING *CYCAS*, IN DELHI, INDIA, AND AN INSIGHT INTO THE NATURE OF THEIR INTERACTION

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Abstract
Larvae of many species of Lycaenid butterflies are known to associate with ants. With respect to larvae, the association can be facultative or obligatory. Also, larvae of some species of Lycaenids maintain a parasitic, and many others a mutualistic relationship with ants. Larvae of *Chilades pandava* (Lepidoptera:Lycaenid:Polyommatini) -a butterfly that has recently extended its range along with the artificial introduction of its larval host plants, several species of *Cycas*, is known to associate with more than one genera/species of ants. We sampled ornamental *Cycas* plants in urban Delhi infested with *C. pandava* for the genera of ants that associate with the larvae of this butterfly. Results compiled from sampling studies in Delhi, and various reports from literature indicated that at least 13 genera of ants can associate with larvae of *C. pandava*. In the present communication, these results have been discussed in light of the nature of association between *C. pandava* and ants.

Keywords Lycaenid-ant Association, Mutualism

Introduction

The larvae of many Lycaenid butterflies are known to associate with ants- 'myrmecophily' (Pierce *et al*., 2002). The relationship between larvae and ants can be parasitic, commensal or mutualistic (Baylis *et al*., 1993; Fiedler, 2012). The latter type of relationship i.e. mutualism, occurs most frequently (Fiedler, 2006a; 2012). In mutualism, the butterfly larvae provide nutritious secretion-'larval nectar' (rich in sugars and amino acids) from Dorsal Nectary Organs (DNO, located in 7th abdominal segment of larvae) to ants. In return, the larvae are exempted from attack by ants and additionally, ants aggressively safeguard larvae from other predators (Pierce *et al*., 2002; Hojo *et al*., 2015; Malicky *et al*., 2005). Larvae also possess a pair of eversible Tentacle Organs (TO), on the 8th abdominal segment which are known to secrete volatile substances, and Pore Cupola Organs (PCO) distributed on abdomen (Pierce *et al*., 2002; Ekka *et al*., 2020). The function of substances secreted from TO is similar to the ant alarm pheromone; it alerts ants when larvae are alarmed, and can communicate the message of need for protection to several species of ants (Pierce *et al*., 2002; Ekka *et al*., 2020). Gnatzy *et al*. (2017) suggested that TO are mechanosensors. PCO is known to secrete substances to appease ants which otherwise may attack soft bodied larvae. Thus, the three organs, i.e. DNO, TO and PCO are important for mutualistic larvae–ant association (Pierce *et al*., 2002; Fiedler, 1991; 1996).

It has been shown that the seemingly mutualistic relationship between Lycaenids and ants is actually of the host-parasite type. Larvae, through certain chemicals in their secretion, can manipulate the behaviour of ants and enforce a cooperative behaviour such as partner fidelity and aggressive defence (Hojo *et al*., 2015; Hughes, 2015).

The association between Lycaenid larvae and ants can be facultative or obligatory (Pierce *et al*., 2002, Fiedler *et al*., 1996; Fiedler, 2012). In the facultative association, larvae do not require or tended by several genera or species of ants, and; larvae are attended by ants only intermittently. In the obligatory association, larvae are invariably associated with a particular genus or species of ants and the survival of larvae is not possible without ants due to high risk of predation or other reasons (Fiedler *et al*., 1996; Pierce *et al*., 2002). In most cases, facultative associations are said to be mutualistic (Pierce *et al*., 2002).

Field data collated for larvae of 435 species of Lycaenids revealed that worldwide, about 53 genera of ants associate with larvae of this group of butterflies (Fiedler, 2001). All the genera of ants reported in the study were also known to forage on honeydew, plant nectar or other liquid sources of carbohydrates. The same study also reported that in India, 21 genera of ants associate with the larvae of 109 species of Lycaenids, with most frequently associated genera of ants being: *Crematogaster* (Lund, 1831), *Camponotus* (Mayr, 1861), *Polyrhachis* (Smith, 1857), *Oecophylla* (Smith, 1860), *Anoplolepis* (Santschi, 1914), *Tapinoma* (Forster, 1850), *Pheidole* (Westwood, 1839), *Paratrechina* (Motschoulsky, 1863) and *Technomyrmex* (Mayr, 1872) (Fiedler, 2001),

Chilades pandava is an invasive butterfly and a serious pest of *Cycas* sp. in some parts of the world (Wu *et al*., 2009;Tennent, 2014;Marler *et al*., 2012; Liu, 2018). It is known to use about 85 species of *Cycas* as host plants including *Cycas revoluta*- the common ornamental Cycad species in gardens (Marler *et al*. 2012). The butterfly lays eggs on nascent fronds of *Cycas* on which the emerging larvae feed. This severely affects the growth of the plant. The larvae of *C. pandava* have been observed to be associated with more than one genus of ants and the association is considered of the mutualistic type (Fiedler, 1991). In the present communication, the ant associations of *C. pandava* have been further studied. Observations made on the *Cycas* plants in urban Delhi (Northern India) revealed association of larvae of *C. pandava* with many more genera of ants. The list of ant genera that can associate with larvae of *C. pandava* has been further augmented by previous reports from various other geographical locations. The results so obtained are discussed in view of the nature of association between *C. pandava* and ants.

Materials and Methods

Observations related to larva-ant association

Thirteen ornamental *Cycas* plants at five different locations (parks and lawns) in urban Delhi (L1-L5, Table 1) were observed for the genera of ants that associate with the larvae of *C. pandava.* All the plants were examined for about 10-15 minutes from top and underside. Ants which were seen in physical contact with larvae were photographed with any of the three mentioned instruments: 1) digital SLR camera (model Nikon-D500), attached with macro lens (AF-S VR Micro-Nikkor 105 mm) or wide angle lens (18-55) with extension tubes; 2) Panasonic GH5 attached with 35 mm lens; and 3) Canon A3200 with 5X zoom. Short duration video clips of ants in physical contact with larva were also made using the above mentioned instruments. The identity of the ants up to the genus level was ascertained through photographs. The information on the numbers of genera of ants associated with larva of *C. pandava* so obtained is given in Table 1.

Literature search

The information on the numbers of genera of ants than can associate with larva of *C. pandava* was augmented by online literature search. The information so obtained is given in Table 2.

Observations related to life cycle of *C. pandava*

Three plants at location L1 (Table 1) were observed for egg laying, larval stage, pupation and eclosion. One breeding cycle of *C. pandava* on each of the three plants was monitored. Observations were commenced from appearance of new fronds till their damage by feeding activity of the larvae and slowing down or culmination of breeding activity of butterflies on the plants. Various stages of *C. pandava* life cycle were photographed (Figure 1).

Results

Breeding activity

Adult *C. pandava* laid eggs on nascent fronds of *Cycas* sp. The discoid eggs of *C. pandava* were greenish-white or pale bluish-white (Figure 1b and c). The majority of eggs hatched within 5-8 days after the fronds were heavily laden with eggs by adults. When the larval population was at its maximum, all the instars could be seen on the plant, possibly due to the asynchronous hatching of eggs of different ages. The emerging larvae fed by boring into the young fleshy fronds as well as on the surface of fronds. On the basis of body colour, larvae could be differentiated into three colour types; pale-green, reddish-purple, and a third form, with an intermediate colour (Figure 1d). Ants were seen in physical contact usually with older larvae. Pupae were observed at the base of leaves after 7 or more days of hatching of the eggs under observation (Figure 1e and f). The breeding activity (egg laying) of *C. pandava* on a plant slowed down and finally stopped after damage to all or most of the nascent fronds by the feeding activity of larvae (Figure 1g).

Observations related to larvae-ant interaction

A total of 6 different genera of ants belonging to 3 subfamilies of Formicidae, i.e. Myrmicinae, Formicinae and Dolichoderinae were found to associate with larvae of *C. pandava* on 13 *Cycas* plants at 5 different locations (L1-L5) in urban Delhi (Table 1 and Figure 2). Ants were observed to make physical contact with the larvae which were about the length of 1 cm or more (older larvae). Ants moved or ran on the dorsal side of larvae with characteristic antennal movements and intermittently paused near DNO (Figure 2) to collect larval nectar. Literature search provided 7 additional genera of ants that associated with larvae at other geographical locations (Table 2).

Further observations made in the present study include: a) on a given *Cycas* plant, not all the larvae seen were in contact with ants during the time span of observation, b) in all but two cases, only a single genus of ants was observed to associate with larvae on a given plant, c) on two occasions, more than one genus of ants were observed to be associated with nearby larvae on the same plant (sharing food site), and d) at location L2 with cluster of 5-6 infested *Cycas* plants, different genera of ants were observed to associate with larvae on different plants at a given time.

Discussion

C. pandava is native to Southern Asia and has invaded many other regions of the world (Wu *et al*., 2009;Tennent, 2014;Liu, 2018;Fric *et al*., 2014: Abu-Shall, 2014;Williams, 2006). It is a serious pest in some parts of the world, threatening various endemic species of *Cycas*, as well as ornamental *Cycas* in gardens (Marler *et al*., 2012; Liu *et al*., 2018). In Delhi, the butterfly profusely lays eggs on budding fronds of *Cycas* from March to November. Extensive damage to the plant is caused by the larvae which feed exclusively on tender fronds.

The larvae of *C. pandava*, particularly the older instars (3rd-4th) are intermittently attended (or tended) by ants (Figures 1 and 2).

The 3rd and 4th instar larvae of butterflies belonging to the tribe Polyommatini are known to possess PCO, DNO and TOs (Fig. 2 g and h) and myrmecophily occurs universally in this tribe (Fielder, 1991; Pierce *et al*., 2002; Ekka *et al*., 2020). The association is of mutualistic type as larvae secrete nectar from DNO in the presence of ants on which the latter feed. And, in response to this food provisioning service, larvae are not only exempted from attack by ants, but are also protected from other predators by the latter (Fielder, 1996; Pierce *et al*., 2002). A total of 13 genera of ants have been found to associate with *C. pandava* larvae (Tables 1 and 2). This includes 6 genera observed in urban parts of Delhi in the present study, and 7 genera from other geographical locations as reported in the literature (Table 1 and 2). Of the 6 genera of ants which we have reported from Delhi, *Meranoplus* have not been reported previously to associate with larvae of *C. pandava*. According to the state-wise checklist of ants in India, Delhi is known to have 19 genera of ants (Bharti *et al*., 2016), and the present study shows that at least 10 of these (6 genera from Table 1 + 4 genera given in bold letters in Table 2) can associate with larvae of *C. pandava*. It is however possible that many more genera of ants that can associate with larvae of *C. pandava* will be discovered if more extensive sampling is done. Since the larvae of *C. pandava* can be tended by several species of ants, and only intermittently, also, larvae can transform into adults even in the absence of any contact with the ants (results not shown), indicates a facultative association between larvae and ants (Fielder, 1991; 1996; Pierce *et al*., 2002) as mentioned by Fielder (2006b).

All the genera of ants that associate with larvae of *C. pandava*, belong to only three subfamilies-Myrmicinae, Formicinae and Dolichoderinae (Table 1 and 2). This observation is in agreement with the previous studies on ant-Lycaenid association (Fielder, 2001; 2006a). The genera of ants that associate with larvae of Lycaenids are known to practice 'trophobiosis'- a phenomenon which requires a complex set of morphological and behavioural traits (Fielder, 2001; 2006a; 2012). Furthermore, in facultative larvae-ant association, ant genera which are ecologically dominant in their habitat or defend territories and monopolize resources, are most likely to be the partner (Fielder, 2001, 2006a; 2012).

It therefore strengthens the view (Fielder, 2006b) that association of larva of *C. pandava* with ants is of mutualistic- facultative type. Furthermore, it is possible that the ability of *C. pandava* larvae to maintain healthy relations with several genera of ecologically dominant and aggressive ants patrolling on *Cycas* plant, and employ these ants as guards against other predators could be a positive feature for the invasive potential of this butterfly. However, to emphasize this notion conclusively, further in-depth studies are required.

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References

Abu-Shall, A., H.M. Ramadan & M.A. Abu-Ghonem. 2014. Immature stages of *Chilades pandava* (Lepidoptera: Lycaenidae), a new pest of *Cycas* spp. in Egypt. *Alex. J. Agric. Res.* 59: 197-204.

Anonymous 1. Preserving biodiversity in Guam's forests: the effect of indigenous and exotic ants on Guam's native trees. Report, USDA, REEIS, Project Director:Miller, R. H., Sponsoring Institution National Institute of Food and Agriculture, Accession No.0191219. https://reeis.usda.gov/web/crisprojectpages/0191219-preserving-biodiversity-in-guamsforests-the-effect-of-indigenous-and-exotic-ants-on-guams-native-trees.html. Accessed on 5.6. 2020

Anonymous 2 http://www.guaminsects.net/gisac2015/index.php?title=Chilades_pandava. Accessed on 5.6.2020

Bingham, C. T. 1907. *Fauna of British India including Ceylon and Burma*. Butterflies, Vol. 2. Taylor & Francis, London. 413–415.

Baylis, M. & N.E. Pierce. 1993. The effects of ant mutualism on the foraging and diet of Lycaenid caterpillars. In: N.E. Stamp & T.M. Casey (eds.), *Caterpillars — Ecological and Evolutionary Constraints on Foraging*. Chapman & Hall, New York/London. 404–421.

Bharti, H., B. Guénard, M. Bharti, & P.E. Evan. 2016. An updated checklist of the ants of India with their specific distributions in Indian states (Hymenoptera, Formicidae). *ZooKeys* 551: 1–83. doi:10.3897/zookeys.551.6767http://zookeys.pensoft.net

Chung, A.Y.C. 2012. Infestation of *Chilades pandava* (Lepidoptera: Lycaenidae) on ornamental cycads and its control measures. *Poster in 5th International Symposium for the Development of Integrated Pest Management for Sustainable Agriculture in Asia and Africa*.18-20 December, 2012, Sutera Harbour Resort, Kota Kinabalu. https://www.researchgate.net/publication/340021059. Accessed on 4.vi. 2020.

Daniels, H., G. Gottsberger & K. Fiedler.2005. Nutrient composition of larval nectar secretions from three species of myrmecophilous butterflies. *J. Chem. Ecol.,* 31 (12) 2805-2821. DOI: 10.1007/s10886-005-8395-y.

Ekka, P.A., S. Kumari & N. Rastogi. 2020.Facultative associations of two sympatric Lycaenid butterflies with *Camponotus compressus* – field study and larval surface ultrastructure. *Halteres* 11: 44-55.

Fiedler, K. 1991. Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenida (Insecta: Lepidoptera: Papilionoidea). *Bonner Zoologische Monographien* 31(1): 1-210.

Fiedler, K., B. Holldobler & P. Seufert. 1996. Butterflies and ants: The communicative domain. *Experientia* 52: 14-24.

Fiedler, K. 2001. Ants that associate with Lycaeninae butterfly larvae: diversity, ecology and biogeography. *Divers. Distrib*.7: 45-60.

Fiedler, K. 2006a. Ant-associates of Palaearctic Lycaenid butterfly larvae (Hymenoptera: Formicidae; Lepidoptera: Lycaenidae). *Myrmecol. News*9: 77-87.

Fiedler, K. 2006b. Digital supplementary material to: 'Ant-associates of Palaearctic Lycaenid butterfly larvae (Hymenoptera: Formicidae; Lepidoptera: Lycaenidae) – a review'. *Myrmecologische Nachrichten* 9: 77-87.

Fiedler, K.2012. The host genera of ant-parasitic Lycaenidae butterflies: A Review. *Psyche.* DOI: 10.1155/2012/153975.

Fric, Z.F., R. Dickinson, G. Fetouh, T.B. Larsen, W. Schön & M. Wiemers. 2014. First record of the cycad blue, *Chilades pandava*, in Egypt – a new invasive butterfly species in the Mediterranean region and on the African continent (Lepidoptera: Lycaenidae). *Afr. Entomol*. 22(2): 315–319.

Gnatzya, W., M. Jatho, T. Kleinteich, S.N. Gorb & R. Hustert. 2017. The eversible tentacle organs of *Polyommatus* caterpillars (Lepidoptera, Lycaenidae): Morphology, fine structure, sensory supply and functional aspects. *Arthropod Structure & Development* 46(6): 788-804.

Hojo, M.K., N.E. Pierce & K. Tsuji.2015. Lycaenid caterpillar secretions manipulate attendant ant behaviour. *Curr. Biol*. 25: 2260–2264.

Hughes, D. P. 2015. Behavioral ecology: Manipulative mutualism. *Curr. Biol*. DOI:https://doi.org/10.1016/j.cub.2015.07.067.

Liu, C. & C. Ping. 2018. Occurrence and prevention and control progresses of *Chilades pandava* in *Cycas* spp. *Plant Diseases and Pests* DOI: 10.19579/j.cnki.plant-d.p.2018.01.001.

Malicky, H. 1970. New aspects of the association between Lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymenoptera). *J. Lepid. Soc.* 24: 190–202.

Marler, E. T., A.J. Lindstrom & L.I. Terry. 2012. *Chilades pandava* damage among 85 *Cycas* Species in a common garden setting. *Hortscience* 47: 1832–1836.

Pierce, N.E., M.F. Braby, A. Heath, D.J. Lohman, J. Mathew, D.B. Rand & M.A. Travassos. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera).*Annu. Rev. Entomol*.47: 733–71.

Tennent, W.J., 2014. First record of the invasive Lycaenid species *Chilades pandava* (Horsfield, 1829), from Papua, New Guinea (Lepidoptera, Lycaenidae)*Nachr. Entomol. Ver. Apollo, N. F.*35(1/2): 43–46.

Williams, A.A.E. 2006. Butterfly observations on Mauritius Island, with notes on three recently introduced Lycaenids, *Leptomyrina phidias* (Fabricius), *Petrelaea sichela reticulum* (Mabille) and *Chilades pandava* (Horsfield) (Lepidoptera: Lycaenidae). *Western Australian Insect Study Society Newsletter* 5-10.

Wu, L.W., D.C. Lees, Y.F. Hsu.2009. Tracing the origin of *Chilades pandava* (Lepidoptera, Lycaenidae) found at Kinmen Island using mitochondrial COI and COII Genes. *BioFormosa* 44: 61-68.

Table 1. Genera of ants found to associate with larvae of *C. pandava* at different locations in Delhi, India**.**

Location	**Plant**	**Genera of ants found to be associate with larvae on different *Cycas* plants sampled**	**Family of ants**	**Total number of ant genera associated with larvae**
L1	P1.1	*Camponotus*	Formicinae	*Camponotus* *Tetramorium* *Meranoplus* *Pheidole* *Crematogaster* *Tapinoma* (Total = 6)
	P1.1	*Tetramorium*	Myrmicinae	
	P1.2	*Camponotus*	Formicinae	
	P1.3	*Camponotus*	Formicinae	
L2	P2.1	*Meranoplus*	Myrmicinae	
	P2.2	*Pheidole*	Myrmicinae	
	P2.3	*Crematogaster*	Myrmicinae	
	P2.4	*Meranoplus*	Myrmicinae	
	P2.5	*Pheidole*	Myrmicinae	
L3	P3.1	*Tapinoma*	Dolichoderinae	
L4	P4.1	*Tapinoma*	Dolichoderinae	
	P4.2	*Tapinoma*	Dolichoderinae	
L5	L5.1	*Camponotus*	Formicinae	

Table 2. Genera of ants associated with larvae of C. pandava at different geographical locations as reported in literature. *The column includes those genera of ants from column 2, not observed in sampling in Delhi. Genera in bold letters in right most column are also found in Delhi India (Bharti et al. 2016)

Location	**Genera of ants reported to be associated with larvae of *C. pandava***	**Family of ants**	**Total number of ant genera associated with larvae at different geographical locations***
Calcutta, India (Bingham, 1907)	Monomorium	Myrmicinae	Monomorium Prenolepis Anoplolepis Iridomyrmex Paratrechina Oecophylla Technomyrmex (Total = 7)
	Crematogaster	Myrmicinae	
	Prenolepis	Formicinae	
Guam (Anonymous 1, and Anonymous 2)	Anoplolepis	Formicinae	
	Tetramorium	Myrmicinae	
	Tapinoma	Dolichoderinae	
	Iridomyrmex	Dolichoderinae	
	Monomorium	Myrmicinae	
	Paratrechina	Formicinae	
	Pheidole	Myrmicinae	
Sabah, Malaysia (Chung 2012)	Oecophylla	Formicinae	
	Anoplolepis	Formicinae	
	Paratrechina	Formicinae	
Unknown (Fiedler 2006b)	Technomyrmex	Dolichoderinae	
Varanasi (Ekka et al. 2020)	Camponotus	Formicinae	

Fig.1*:* Various stages in the life cycle of *C. pandava* as observed on *Cycas* sp. at location L1. a) Mating adults, b) nascent leaf dotted with *C. pandava* eggs, c) close up of eggs, d) larvae on infested frond e) final larval instar at the site of pupation, f) final larval instar and pupa, g) *Cycas* plant with fronds damaged by *C. pandava* infestation.

Fig.*2*: Various ant genera associated with the larvae of *C. pandava* in Delhi, India. a) *Tapinoma*, b) *Tetramorium*, c) *Camponotus* d) *Pheidole* e) *Meranoplus*, and f) *Crematogaster*, g) showing response of *C. pandava* larva after interaction with ant (*Camponotus* sp.); encircled portion showing Dorsal Nectary Organ (DNO) with oozing drop of liquid, and eversible pair of Tentacle Organs (TO), h) close up of DNO and TO shown by arrows '1' and '2' respectively.