Strange bedfellows: How butterfly caterpillars sustain their association with cocktail ants

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Association of caterpillars of Blues and Hairstreaks (Lycaenidae) with ants range from absent to facultative (opportunistic) to obligate (compulsory), and from mutualistic to parasitic. At one end of the spectrum, caterpillars of species such as the Red Pierrot (Talicada nyseus; left) do not associate with ants directly, but are also not attacked by them as are most caterpillars. They have thick larval skins or cuticles characteristic of many lycaenid caterpillars, and appear to retain pore cupolae organs, but all other ant associated structures develop only to a rudimentary state. Caterpillars of facultative associates such as the Common Cerulean (Jamides celeno; centre) live with ants whenever they find the right ant species, but they are also fine without ants. In these species, the ant-associated organs are developed reasonably. At the other extreme, caterpillars of species such as the Lilac Silverline (Apharitis lilacinus, right) are obligate associates of ants, in which the ant-associated organs are remarkably developed. Females of such obligate associates lay eggs very close to ant nests. Caterpillars and pupae are constantly attended by ants, and they may live inside ant nests, often among the brood of ants. In some of these species, caterpillars do not feed on plants at all. Instead, they eat food regurgitated by the tending ants. In a rare turn of events, the caterpillars of some species may also slyly eat the ant brood, turning this association a bit sinister. Credit: Krushnamegh Kunte

The spectacular leaps of gazelles, group living in deer and monkeys, and fast flight in many insects are all linked by a common phenomenon—predation. In its various forms, predation has driven the evolution of a plethora of specialized structures (morphology) and behaviors among organisms. Insects, being especially vulnerable because of their small size, have evolved various strategies to avoid predators. For example, butterflies may either accumulate toxins (aposematism), mimic other toxic species (mimicry), avoid detection by predators by remaining inconspicuous (crypsis or camouflage), or look like inedible plant parts (masquerade) to escape predators.

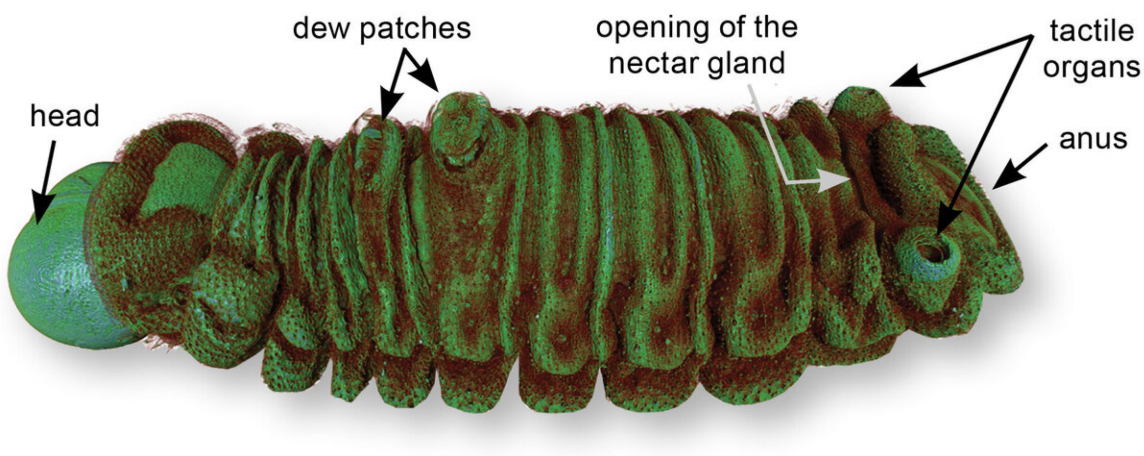
Butterflies of the family Lycaenidae, popularly known as Blues and Hairstreaks, have gone in a completely unexpected direction to deal with their predators. Caterpillars and pupae of the majority of the approx. 5,200 lycaenid species do not avoid predatory ants at all. In fact, they seek and closely associate with ants, becoming strange bedfellows. Ants not only do the ants not eat these caterpillars and pupae, but they actually care for them and aggressively protect them from other predators and parasitoids, thus creating an enemy-free space. Many of these associations have been perfected over millions of years via an evolutionary arms-race between the caterpillars and ants. How are these strange associations between predator and potential prey species sustained?

The lycaenid caterpillars are far from vulnerable in this association. Over tens of millions of years of evolution, this butterfly group has evolved a range of adaptations that have tamed their ferocious ant predators into protectors and providers. Lycaenid caterpillars typically have at least four types of specialized organs that produce chemical concoctions that modulate the nature of their ant associations, ranging from facultative to obligate, and mutually beneficial to behaviourally manipulative and parasitic. First, the body surface of lycaenid caterpillars has clusters of pheromone-secreting glands called pore cupolae. Pore cupolae are thought to secrete chemicals that secure favorable recognition by the ants, thereby subduing their aggression.

Next, two other organs, called dew patches in some species and nectar glands in others, produce carbohydrate-rich secretions to attract and reward the tending ants. For the most part, these sugary secretions, which ants drink readily, keeps the ants interested in tending and protecting the caterpillars. Ants can sometimes be seen stroking the caterpillars with their antennae to encourage them to produce these secretions, and the caterpillars often comply. So this association can be beneficial for both parties. However, caterpillars can sometimes deceive the ants by luring them with these secretions but then reabsorbing the secretions before ants can take them. One supposes that the caterpillars have to juggle between keeping the tending ants happy so that they continue to receive protection, while minimizing the energetic cost of producing these nutritious secretions. Who isn't tempted to skim off some profits from business partners once in a while?

In some species, the secretions of dew patches and nectar glands have been shown to alter the levels of neurotransmitters, particularly dopamine, in the brains of their attendant ants, causing them to slow their locomotory activity. This also makes the ants more faithful to the caterpillars and increase the level of aggression toward their parasites and predators. In cases like these, it is better to consider these apparently mutualistic interactions to be reciprocal parasitisms, where natural selection may turn the strategies used by each partner to outwit the other into a sophisticated coevolutionary arms race.

Finally, caterpillars deter the ants by rhythmically everting a pair of tentacle organs or tactile organs when the density of tending ants increases too much, or when the caterpillar wants to move. Ciliary tufts at the tips of these organs also contain receptors of ant pheromones, and act as a compass to direct caterpillars toward ant aggregations.



A false-colour MicroCT reconstruction of the Lilac Silverline caterpillar (Apharitis lilacinus), showing the placement of its specialized ant-associated organs. Credit: Dipendra Nath Basu

Additionally, caterpillars may produce substrate-borne vibrations to draw the attention of specific species of ants. In some species, caterpillars have evolved to mimic the characteristic smell, sounds and certain behaviors of ant larvae and even ant queens. These incredible adaptations mean that worker ants sometimes carry their caterpillar guests inside the nests, keeping them among their brood, caring for them and feeding them like their own.

Thus, these caterpillars manipulate ant behavior with multimodal signals involving chemical, acoustic, and tactile means. These modes of interaction are deployed selectively in specific interactions with ants. Together, these ant-associated organs and behaviors of caterpillars orchestrate a 'push and pull' mechanism to manipulate ant behavior to the advantage of caterpillars.

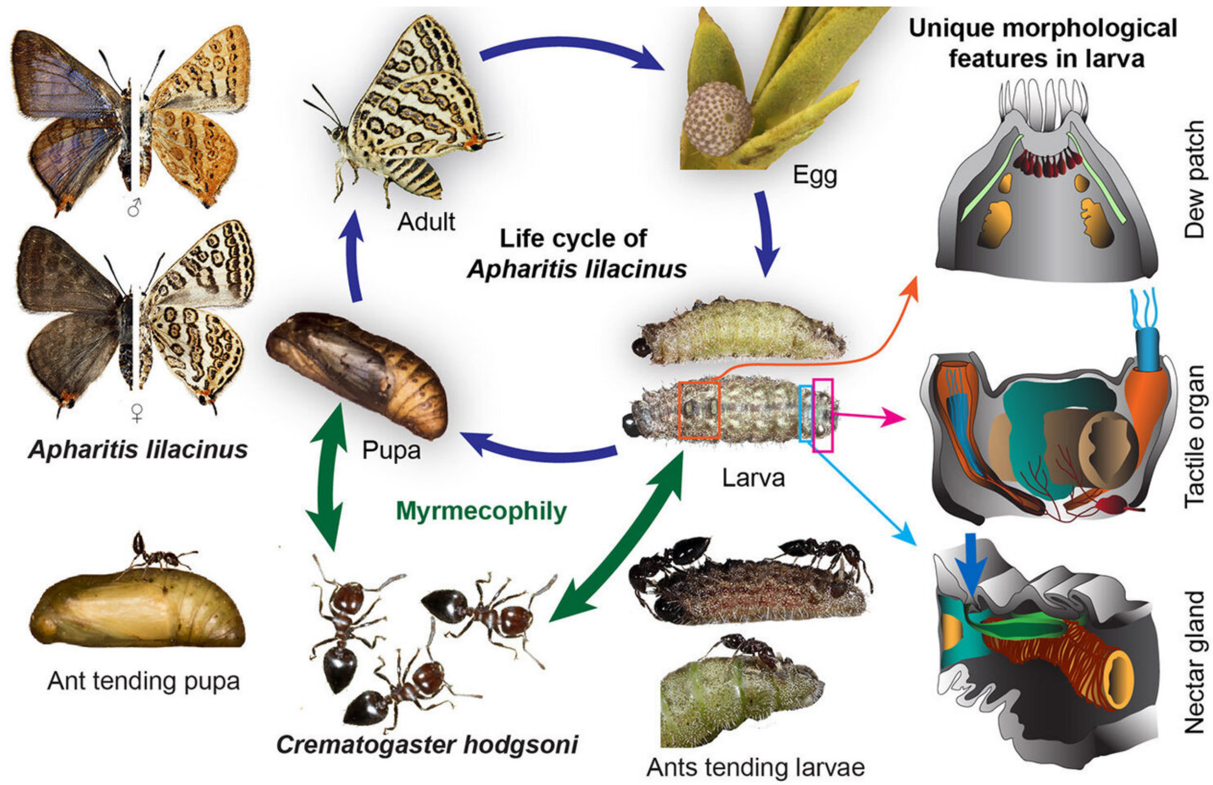
These fascinating caterpillar-ant associations have been studied for many decades, generating a detailed understanding of their evolution and ecology. The presence of the ant-associated organs of these caterpillars, variation in their positions on the body, and their relative development with respect to the nature of ant associations are also well known. However, how these organs actually work (i.e., their functional morphology) has been poorly understood. This is because the native structures of these organs inside the body and their relative positions with connecting muscles and nerves get destroyed in traditional methods of dissection and staining. It is difficult to understand the functional significance of these organs and associated muscles and nerves when they are out of their native context inside the body.

Dipendra Nath Basu, a Ph.D. student, and his advisor Dr. Krushnamegh Kunte at the National Centre for Biological Sciences, Bengaluru, found this problem both peculiar and fascinating. To find out how these critically important organs function in sustaining these caterpillar-ant associations, they turned to X-ray microtomography, or MicroCT (remember that CT-scanning is used widely in hospitals). MicroCT has recently emerged as a valuable tool for studying organ development and functional morphology of smaller animals such as lizards and insects. Using MicroCT, we can map the entire internal structure of the body in great detail, without the need to dissect and disrupt the organs in their native states. Also, with a single scan, any structures inside the body can be studied either in isolation or in relation to other structures. This is precisely the kind of technology that can shed light on the functional morphology of the ant-associated organs, so Dipendra got busy mastering it.

Around the same time, another set of naturalists was occupied with studying the natural history and ecology of a rare species of butterfly in the Bengaluru area. Nitin Ravikanthachari, still an undergraduate student, had rediscovered a population of the Lilac Silverline (Apharitis lilacinus) a few years earlier. This butterfly had not been seen in India for over a hundred years, but Nitin stumbled upon it while photographing butterflies at Hesaraghatta lake. His subsequent observations revealed that this suburban wildland had a stable breeding population of this butterfly. Excited by this rare discovery, Nitin, along with fellow naturalists Ashok Sengupta and Girish Kumar G. S., started making regular visits to Hesaraghatta, learning everything they could about the biology of this species.

They soon discovered that the Lilac Silverline caterpillars have an obligate association with a single species of cocktail ant (named so because they often hold their heart-shaped gasters or 'tails' up when they are alarmed), called Crematogaster hodgsoni. Females of the Lilac Silverline deposit eggs at the entrance of cocktail ant nests, sometimes on sand and away from plants. Caterpillars are completely dependent on ants after hatching from eggs, and they are constantly attended by their hosts. Indeed, they live inside the ant nests, often among the ant broods, and are cared for by the tending ants just like the rest of their own brood. As far as known, caterpillars exclusively feed on regurgitated food provided by ants; they have not been observed so far feeding on plant tissue, like most other caterpillars do. As expected from their close relationship, the caterpillars possess all the main ant-associated organs that have been described in other obligate ant-associates, and these organs are very well developed in this species.

Dipendra took advantage of this fascinating species occurring in close proximity of Bengaluru. His MicroCT scan of the caterpillar provided high resolution and three-dimensional reconstruction that enabled the detailed characterization of hard as well as soft tissues such as muscles and nerves in their native states inside the body. This revealed the functional morphology of the ant-associated organs of this caterpillar in unprecedented detail. The MicroCT scan revealed how surrounding muscles may contract and relax, enabling the caterpillar to control the release and reabsorption of secretory droplets from dew patches and nectar glands that lure and sometimes deceive ants. Dew patches, for example, operate on a 'lasso bag' control mechanism using surrounding muscles. Dew patches are assemblages of multiple gland lobules opening in a common cavity, which is guarded above with an external orifice resembling a bag/sac in cross-section. The cavity and the orifice on their inner surfaces are attached to sets of retractor muscles that control the opening and closing of dew patches similar to a lasso. The lasso bag mechanism is known to operate glands in a few other organisms as well, and its use in dew patches makes sense since it offers a greater control over the release and reabsorption of secretions. The scans also suggested how muscle action and haemolymph pressure, controlled by abdominal ganglia, might cause the rhythmic motions of the tactile organs.



The lifecycle of the Lilac Silverline butterfly (Apharitis lilacinus) showing larval and pupal associations with Crematogaster hodgsoni cocktail ants, a behaviour that is termed myrmecophily, meaning 'love of ants'. Structures of the specialized organs that mediate caterpillar-ant interactions are shown on the right. Taken from Basu and Kunte (2020). Credit: Images of egg: Nitin Ravikanthachari; pupa and ant-caterpillar/pupa interaction: Ashok Sengupta and G. S. Girish Kumar; larval organs: Dipendra Nath Basu; specimens: Krushnamegh Kunte

Since the nutrient-rich rewards and other chemical investments aimed at keeping the ants engaged are energetically likely very expensive, caterpillars must produce and release them judiciously. Naturally, the best way to keep your partners engaged is to let them know about the potential rewards, but then dish out the expensive good stuff in small doses in a controlled manner. The detailed insights into the functional morphology of the ant-associated organs and the mechanisms of gland operations indicate how caterpillars may be able to have a fine control over when they reward ants, and how much, thus optimizing their investments and returns.

MicroCT scans also revealed additional adaptations of these obligate ant-associates. The caterpillars have a thick skin or 'dermis' with chitinous thoracic and abdominal plates, which shield their front and rear ends from ants should they become momentarily aggressive. Internal morphology also revealed a narrow foregut missing most of the musculature that is required in caterpillars of other species to digest tough plant tissue. Since the Lilac Silverline caterpillars only eat food regurgitated by ants, which is presumably easily digested, they have largely lost the musculature around the foregut. On the other hand, even in a detailed MicroCT analysis, the team could not detect any special morphological features in the pupa that might facilitate its association with ants. It is possible that the pupae maintain their close association with ants purely via chemical signals.

Prof. Naomi Pierce of Harvard University, a leading expert on caterpillar-ant associations who was not involved in this study, is impressed and excited about these findings. "This work opens up the wonderful world of caterpillar-ant associations for Indian biologists and naturalists," she remarked, observing that the vast number of Blues and Hairstreaks that occur in India should provide ample opportunities to study not only the diversity of caterpillar structures and behaviors, but also their species-specific associations with ants that might have evolved over millions of years across India's biodiversity hotspots.

"While caterpillar-ant interactions have been explored as fine examples of multi-partner interactions, i.e. between caterpillars, ants and potential predators, what is important and novel about this study is the study of the mechanisms that facilitate these fascinating interactions," agreed Prof.

Renee Borges of the Centre for Ecological Sciences, Indian Institute of Science, who is an expert on plant-animal and other inter-specific interactions. She continued, "It will be very exciting to study how the evolutionary arms race between ants and caterpillars has shaped the functional nature of the interactions between these vastly different insects, including adaptions and counter-adaptations in anatomy, physiology, behavior and chemistry. What determines the fine balance between mutualism and exploitation in these close associations? A new generation of evolutionary biologists in India will have to find out."

"Guess we have a lot of exciting work cut out for us," Dipendra and Nitin say with a smile. They are looking forward to diving further into the dynamics of evolution of interspecific interactions. "We are incredibly fortunate to be able to inspire young people to find out something very cool and fascinating about the superb biodiversity and nature around us," said Dr. Kunte, with a touch of pride for his students.

Provided by National Centre for Biological Sciences