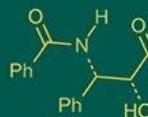


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Nature's Tandem-Tango: Mutual marvel of acacia ants

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Abstract

Myrmecophily encompasses the beneficial interspecies interactions between ants and various organisms, including arthropods and plants, facilitating a diverse range of ecological relationships. Among these, the interactions between the bullhorn acacia (*Vachellia cornigera*) and the acacia ant (*Pseudomyrmex ferruginea*) exemplify a highly developed obligate mutualism. Bullhorn acacias exhibit unique physical traits, such as swollen thorns, extrafloral nectaries, and Beltian bodies, which collectively foster a symbiotic relationship with acacia ants. In return for shelter and food, the ants provide essential protection against herbivores and aid in seed dispersal, enabling the acacia to achieve reproductive success. This interdependent relationship dramatically influences the evolutionary adaptations of both species, as the acacia's ability to thrive relies on the presence of its ant partners, while the ants are similarly dependent on the acacia for critical resources. Research indicates that the removal of acacia ants from their host trees leads to significant detrimental effects on acacia growth and survival. Furthermore, the dynamics of resource allocation and defensive strategies in this mutualism illustrate the complexities of coevolution, where both species continuously adapt to optimize their shared benefits, underscoring the importance of mutualistic interactions in shaping ecological outcomes. This study ultimately highlights the intricate connections within ecosystems that foster species interdependence and evolutionary change.

Keywords: Myrmecophily, bullhorn Acacia Ants, swollen thorn, extrafloral nectaries, beltian bodies

1. Introduction

Myrmecophily refers to the beneficial interspecies interactions between ants and various other organisms, including arthropods, insects, and plants. Though typically associated with mutualistic relationships, the term can also apply to commensal or even parasitic interactions involving ants.

Many arthropod species, particularly mites, rely on ants and live within their nests, giving an example of Ant-Arthropod interactions. Mites are especially proficient as myrmecophiles because their small size allows them to infiltrate ant nests and avoid being expelled. Studies have shown that mites demonstrate an exceptionally high degree of myrmecophily compared to other myrmecophiles^[21]. Ant-insect interactions is one of the most extensively studied myrmecophilous relationships involving ants and hemipterans, especially aphids. Aphids feed on plant phloem sap and excrete honeydew droplets, which ants consume. The ants then return to their nests and regurgitate the honeydew for their nestmates^[11]. Similarly, Ant-plant interactions are widespread across various geographical regions, with numerous species of myrmecophytic plants found in families such as Leguminosae, Euphorbiaceae, and Orchidaceae. These myrmecophytes typically provide ants with shelter and food in exchange for "tending" services, which may include protection, seed dispersal (myrmecochory), reduced plant competition, hygienic services, and nutrient supplementation^[8].

The evolution of myrmecophilous interactions has played a significant role in the ecological success of ants, providing them with a consistent and energy-rich food source, which gives them an advantage over other invertebrate predators^[11]. Myrmecophytes are plants that engage in a mutualistic relationship with ant colonies, where both species benefit from each other's activities. There are over 100 different genera of myrmecophytes worldwide, particularly in tropical regions^[22]. These plants have developed structural adaptations in their rhizomes, leaves, or stems, providing food and shelter for ants. In return, ants assist with

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pollination, seed dispersal, nutrient collection, and defense. The mutualistic relationship between ants and myrmecophytes can be either facultative or obligate [24].

In facultative mutualism, the survival of both parties (plants and ants) does not depend on the interaction. Both organisms can survive independently. This type of mutualism is common in plants with extrafloral nectaries but no specialized structures for ants [20]. These nectaries allow multiple animal species to interact with the plant. Facultative relationships can also develop between non-native plant and ant species, even in the absence of co-evolution. For instance, Old World legumes introduced to North America can be protected by ants from different regions. On the other hand, the obligate mutualism, where both organisms are interdependent and cannot survive without each other. An example of obligate ant-plant mutualism is seen in Bullhorn acacias (*Vachellia cornigera*) and their *Pseudomyrmex* ants in Central America [12].

Daniel Hunt Janzen, an American evolutionary ecologist and conservationist, studied this system of obligate ant-plant mutualism in the late 1960s. He provided some of the earliest experimental evidence showing that ants significantly reduce herbivory rates in myrmecophytes. In September 1966 [13], Janzen published a review paper titled "Coevolution of a Mutualistic System in New World Tropics between Species of Acacia (Mimosoideae; Leguminosae), *Acacia cornigera*, and the Ant *Pseudomyrmex ferruginea* (Formicidae)," where he explained the interdependence between swollen-thorn acacias and their ant inhabitants. In the neotropics, *Acacia* species are protected by ants from defoliation, and in return, the ants are rewarded with specialized organs and physiology that the *Acacia* has evolved.

2. Bull horn acacia (*Vachellia cornigera*)

Vachellia cornigera, also known as bullhorn acacia and belonging to the Fabaceae family, is one of 12 species of swollen-thorn acacias native to tropical regions ranging from the coasts of Mexico to Colombia [19]. Among these species, *V. cornigera* is particularly well-researched, with its distribution, morphology, and ecology extensively documented by Daniel Janzen. Bullhorn acacias can grow to heights of 6-18 meters and have a lifespan of 15-35 years [19]. Their bark ranges from gray to brown and features small furrows. The new growth on their branches is reddish-brown and covered with fine hairs, or pubescence. The leaves of the plant are alternately arranged, with each leaf attached to the branch by a pair of stipular spines [1]. The color of these spines can vary widely, including shades of brown, red, and yellow. Swollen-thorn acacias are set apart from other acacia species by several unique features [10] such as, large, hollow thorns known as "swollen" thorns, modified leaflet tips on young leaves, referred to as beltian bodies, Extrafloral nectaries (EFN) located on their petioles and continuous leaf production throughout the year.

These distinct traits are primarily adaptations for their mutualistic relationship with acacia-ants. Ant colonies reside in the hollow thorns and feed on the nectar produced by the extrafloral nectaries as well as the Beltian bodies [19]. The nectar provides carbohydrates for the worker ants, while the Beltian bodies offer a mix of carbohydrates, lipids, and proteins for the ant larvae [10]. In return, the acacias rely on their ant inhabitants for protection against herbivores and encroaching vegetation, as they are unable to reach

reproductive maturity without the presence of these ant colonies. *V. cornigera*, unlike some other plants, does not use chemical compounds for herbivore defense [17].

The survival of acacia-ants is equally dependent on the swollen-thorn acacias, as they rely on the acacias for both food and shelter. This interdependence makes their mutualistic relationship obligate, meaning that neither the ants nor the acacias can survive without the other. *V. cornigera* has the broadest geographic and ecological range among the swollen-thorn acacias, thriving in a variety of soil types and even in the driest environments. It often coexists with *V. collinsii* or *V. hindsii*, depending on the moisture levels of the habitat [18].



Plate 1: *Vachellia cornigera* with spiny thorns. (Source: Wikipedia)

3. Acacia ant (*Pseudomyrmex ferruginea*)

One ant species that has established an obligate mutualistic relationship with *V. cornigera* is *Pseudomyrmex ferruginea*. This mutualistic relationship involves the reciprocal exchange of resources and services between the two species, benefiting both partners in terms of fitness [2]. The genus *Pseudomyrmex* comprises over 200 species, approximately 40 of which engage in symbiotic relationships with myrmecophilous plants [3]. *P. ferruginea*, commonly known as the acacia ant, is characterized by its wasp-like appearance, with an orange-brown body about 3 mm in length and notably large eyes. Within the *P. ferruginea* group, also referred to as the "ferrugineus complex," there are 10 species that form obligate mutualisms with swollen-thorn acacias [27]. These species are: *P. ferruginea*, *P. flavicornis*, *P. janzeni*, *P. mixtecus*, *P. nigrocinctus*, *P. particeps*, *P. peperi*, *P. satanicus*, *P. spinicola*, and *P. veneficus*. Speciation within this group has been influenced by geographic isolation, with different ant species evolving mutualistic relationships with various swollen-thorn acacia species across shared geographic areas [28].

Many species in the complex, including *P. ferruginea*, can nest in multiple *Vachellia* species. *P. ferruginea* has a broad distribution extending from southern Mexico to El Salvador and Honduras and has been observed in association with all *Vachellia* species within this range, including *V. chiapensis*, *V. collinsii*, *V. cookii*, *V. gentlei*, *V. globulifera*, *V. hindsii*,

V. janzenii, *V. mayana*, *V. sphaerocephala*, and *V. cornigera* (6; 15). In addition to *P. ferruginea*, *Vachellia cornigera* can be colonized by several other species within the *P.*

ferrugineus group, such as *P. nigrocinctus*, *P. veneficus*, *P. peperi*, *P. mixtecus*, *P. flavicornis*, and *P. spinicola* ^[6].



Plate 2: Acacia ant (*Pseudomyrmex ferruginea*) (Source: Wikipedia)

4. Acacia traits related to the ant-acacia coevolution

4.1. Year-round leaf production

In a population of swollen-thorn acacias, many plants maintain green leaves throughout most of the year, or nearly so. These acacias are notably the last to shed their leaves during exceptionally dry periods. In contrast, other acacia species typically lose their leaves and cease leaf production during the latter three-fourths to one-half of the dry season. Although swollen-thorn acacias experience minimal vertical growth during these dry times, they continue to produce small axillary tufts of leaves on flower-bearing shoots ^[26]. These leaves, which have beltian bodies and fully developed nectaries but lack swollen thorns, are crucial for sustaining the ant colony. Without these leaves, the ants might face starvation or be forced to migrate to other acacias with available foliage. In the dry season, the acacia depends on its ant colony for three primary purposes such as, shielding the newly emerging flowering branches, defending the woody parts of the plant from rodents and beetles that bore into wood and maintaining a robust ant colony that will be ready and thriving when the rainy season begins ^[13].

Ant colonies exhibit some flexibility in their dependency during severe dry periods. They can survive for three to five weeks without significant losses, and parts of the colony may temporarily relocate to other acacias that still have leaves ^[3]. Within a given species of swollen-thorn acacia, there is genetic and age variation in the timing and extent of leaf drop. Suckers and seedlings under one year old typically retain their leaves, which is crucial for the survival and establishment of new colonies by founding queens. Even when stumps are cut or burned during the driest period, they quickly sprout new suckers, allowing either existing colonies to relocate or new colonies to establish.

Seeds, however, do not germinate during the dry season. Some mature swollen-thorn acacias may retain their leaves and continue producing new axillary tufts for up to a month longer than others. When a colony occupies multiple acacias, it tends to consolidate in the plant that holds it leaves the longest. This plant generally performs better at the onset of the rainy season, as it supports a higher density of workers ^[13]. The queen and the majority of workers

remain in this acacia, which typically has more workers compared to those abandoned during the dry season. Thus, there is a clear evolutionary advantage to retaining leaves, despite the physiological challenges of doing so during severe droughts.

4.2. Stipules longer persistent, woody with soft pith

Many New World acacias are characterized by woody stipules that vary in length and are often tipped with sharp points. In swollen-thorn acacias, these stipules are notably thickened and elongated, which is essential for their function as domatia—small chambers created by plants to house arthropods like ants. These thorns persist on the acacia for two to four years, though after one or two years, ants no longer find them suitable as domatia ^[1]. The internal volume of these thorns can range from 0.1 to 7.0 cubic centimeters, with the entire canopy of a swollen-thorn acacia typically containing 100 to 800 cubic centimeters of internal thorn space. This space is crucial for housing the ant colony, including both the brood and many of the worker ants. The ants make an entry point by cutting a hole in one side of the thorn, usually near an apex, while the thorn is still green and fully expanded. They then excavate and remove the soft, sweet parenchyma from inside the thorn. These thorns provide essential protection, especially during the dry season when other food sources for the ants are scarce ^[3]. The tough, hard walls of the thorns help shield the ants from predators like birds. However, thorns with thinner walls can sometimes be breached, which can be disastrous if the colony's queen is inside, potentially leading to the colony's collapse.

The development of spines as a deterrent to herbivores has been a subject of sporadic interest in scientific literature. Spiny plants, such as many acacias, are commonly found in arid regions where green foliage is limited during certain parts of the year. While these plants are not entirely immune to browsing, the spines likely reduce their appeal to herbivores, placing them lower on the list of preferred food sources. It is plausible that the evolution of swollen thorns from ordinary stipular spines was driven by the need to support larger, more stable colonies of obligate acacia-ants.

These ant colonies, in turn, likely favor acacias with better-developed thorns, promoting the survival of those plants. In some other acacia species with relatively large thorns, acacia-ant queens may attempt to excavate a cavity, but they often find the internal pith too hard and the thorn wall too tough, even when the thorn is still green ^[1]. For the acacia, having the entire ant colony housed within its canopy is advantageous, and this requires a significant volume of suitable domatia. While some prototype acacias have thorns that can serve as domatia, they do not produce enough of them to support a colony large enough to patrol the entire tree.

The ant colony residing in the swollen thorn acacia's canopy faces various environmental challenges, including exposure to sunlight, bird predation, rain, and desiccation. Consequently, the thorn walls must be waterproof and resilient to sudden temperature changes. Even after a fire hot enough to kill the shoot, the ant colony often survives and relocates to new shoots emerging from the rootstock ^[30]. Despite the sharp tips of the swollen thorns, they do not fully protect the long leaves of the acacia, which are larger and longer than those of most other acacias. Browsing mammals, such as cattle and deer, can easily strip the leaves from unoccupied branches without encountering the thorns.

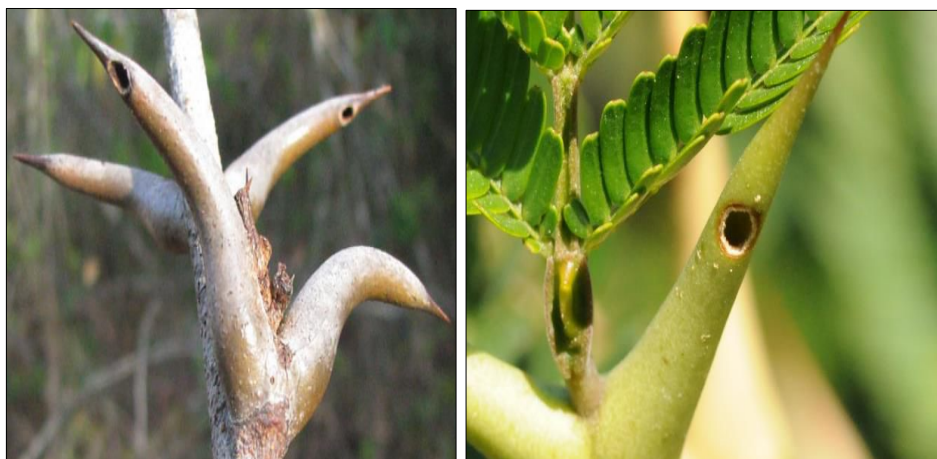


Plate 3: Spiny thorns modified to Domatia and inhabited by mutualistic ants. (Source: Backyard Nature)

The differentiation of Acacia tree species based on their interactions with mutualistic and non-mutualistic ants, as well as how these species manage their rewards, remained unclear until recent studies. One notable study by Amador-Vargas *et al.* in 2020 ^[1] specifically examined the impact of mutualistic versus parasitic ants on the spine characteristics of *Vachellia collinsii*, a species of acacia tree. The study provided insights into whether the number and size of swollen spines on these trees varied according to the level of

protection offered by the ant partner. At the study site in Palo Verde, three parasitic ant species were observed: *Crematogaster crinosa*, which nests inside the spines and consumes nectar, and *Pseudomyrmex nigropilosus* and *P. gracilis*, which also consume plant-produced food bodies. These parasitic ants typically colonize large trees that were previously inhabited by mutualistic ant colonies, and *C. crinosa* is even capable of displacing mutualistic ants from mature trees.

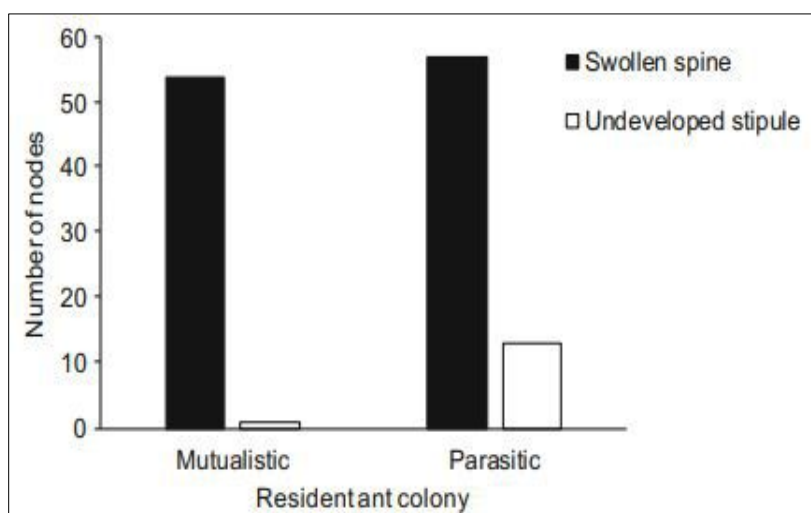


Fig 1: The count of nodes on *Vachellia collinsii* trees where stipules either formed into swollen spines or remained undeveloped, depending on whether the trees were inhabited by mutualistic or parasitic ants.

To determine if tree rewards, such as spine size and the number of nectaries per leaf, were correlated with the type of ant interaction, several parameters were measured. These included the spine size, the diameter at the tree's trunk base

(5 cm above the ground), and the number of nectaries per leaf. Each tree node has the potential to develop a leaf and a spine, which could further develop into domatia and nectaries depending on the type of ant interaction. The

study's findings showed in below graphs revealed that on trees inhabited by parasitic ants, 18% of 70 nodes lacked swollen spines, compared to just 1.85% of 55 nodes on trees defended by mutualistic ants. Additionally, the spines on parasite-inhabited trees had a smaller average diameter by 0.19 cm, though the spine lengths were similar between trees with different types of ant inhabitants. As a result, the spines of trees defended by mutualistic ants had twice the volume of those on trees inhabited by parasitic ants.

However, the variation in the number of nectaries per leaf was not influenced by the type of ant. Overall, the study showed that *V. collinsii* trees inhabited by parasitic ants had fewer swollen spines, smaller spine diameters, and reduced spine volumes compared to those inhabited by mutualistic ants. This suggests that acacia trees adjust their rewards, particularly in the form of shelter, in response to the level of defense provided by their ant partners, limiting resources when they sense a lack of protection [1].

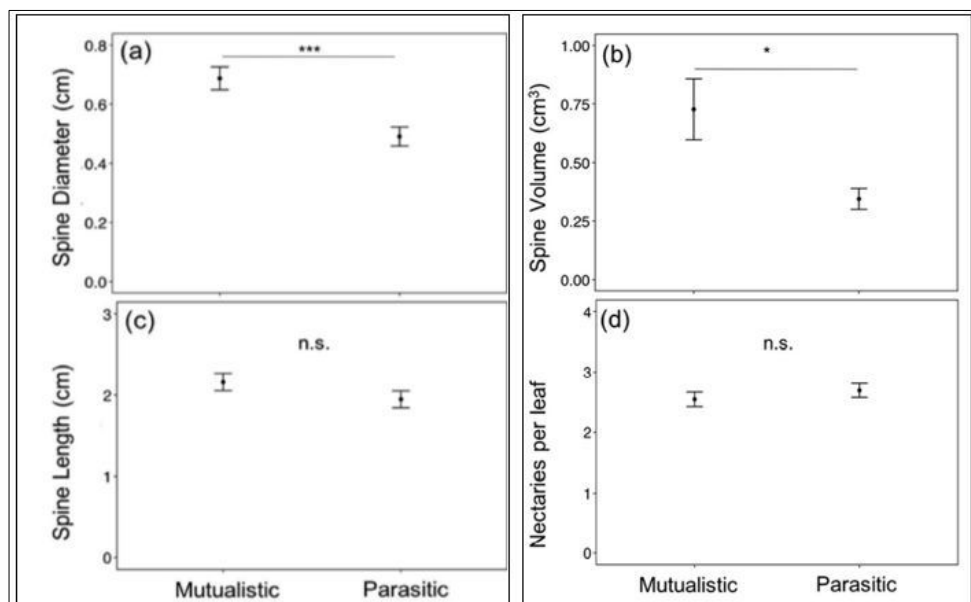


Fig 2: The rewards assessed in *Vachellia collinsii* inhabited by mutualistic versus parasitic ants, focusing on spine length, spine diameter, spine volume, and the number of nectaries. per leaf.

4.3. Highly Developed Foliar Nectaries

Foliar nectaries, located at dorsal part of the petiole or rachis of all mature leaves, are nectar-producing glands that vary in length from 0.5 to 6 mm. In swollen-thorn acacias, these nectaries are notably larger and generate a more substantial flow of nectar compared to other acacia species. During a 24-hour period, larger nectaries can produce a 2 mm sphere of clear, sticky nectar, with the most intense flow occurring about an hour before sunrise. This nectar attracts a diverse group of predatory and parasitic insects, including cockroaches, beetles, Hymenoptera, and flies. The presence of these insects, particularly ants, are significant as they often begin their predatory activities near the nectar site and then spread outwards, making the plant a focal point for searching for phytophagous insects [7].

The nectar is composed of roughly equal amounts of sucrose and fructose, with a concentration of about 40 mg/cc H₂O, providing a reliable sugar source for ant species. A two-meter-tall acacia can produce approximately 1 cc of nectar daily, equating to around 40 mg of combined glucose and fructose. It is suggested, the evolution of foliar nectaries played a significant role in attracting the ancestral *Pseudomyrmex* ants to the ancestral swollen-thorn acacias, highlighting the importance of nectar production in the coevolutionary relationship between ants and acacias. Extrafloral nectar (EFN) serves as a key component of a plant's indirect defense mechanism by attracting protective ants. However, like all mutualistic rewards, EFN is susceptible to exploitation by non-ant consumers that do not contribute to the plant's defense against herbivores. It is important to understand how plants allocate energy towards

EFN production, especially under challenging environmental conditions. In a previously discussed study, it was observed that the efficiency of these rewards diminished when parasitic ants were present [13].



Plate 4: Acacia ant collecting Extrafloral nectar (EFN) (Source: Backyard Nature)

In their 2012 study, Gonzalez-Teuber *et al.* [7] examined how mutualistic plants, specifically two species of Acacia (*V. cornigera* and *V. hindsii*), adjust their reward mechanisms to attract more protective ants and counteract

EFN thieves. Conducted across both rainy and dry seasons, this field experiment analyzed the relationship between EFN secretion and the biotic and abiotic stresses faced by these acacias. The study revealed that EFN production was notably higher in dry season when compared to the rainy season in both *Acacia* species. Correspondingly, ant activity increased during the dry season [12]. The research also found that the level of ant aggression towards nectar thieves was influenced by the concentration of nectar. Branches treated with a higher sugar concentration (20%) saw significantly more ant attacks on EFN thieves than those treated with a lower concentration (5%). Additionally, the time EFN thieves spent on *V. cornigera* nectaries decreased as nectar concentration increased, suggesting that higher rewards not only attract more protective ants but also more effectively deter nectar thieves [17]. This study offers valuable insights into the adaptive strategies of mutualistic plants in response to environmental stresses, highlighting the complex interactions between plant defenses, ant behavior, and seasonal changes. The level of investment in EFN secretion by these plants is positively correlated with the beneficial effects provided by acacia ants.

4.4. Leaf tip modified into Beltian bodies

Acacias often feature twice-compound leaves with a large number of leaflets, typically ranging from 500 to 2000. However, it is only in the swollen-thorn acacias, or those believed to be genetically related, that the tips of these leaflets are modified into specialized structures known as Beltian bodies. Beltian bodies are detachable tips on the pinnules of these acacias and are rich in lipids, sugars, and proteins, often exhibiting a red coloration (13). Named after Thomas Belt, these structures are thought to have evolved as part of a symbiotic relationship with ants. Beltian bodies consist of large, thin-walled cells filled with cytoplasm and nutrients, including proteins, fats, and vitamins.

Interestingly, German cockroaches can grow nearly as well on a diet of Beltian bodies as they do on yeast. Ants harvest these bodies shortly after they fully expand on new leaves, cutting them into pieces to feed their larvae. Although adult worker ants may derive some nutritional advantages from the fluids released during this process, Beltian bodies, in conjunction with nectar, constitute the main food source for the obligate acacia-ant colony. It is only on rare occasions that these ants capture small insects, utilizing parts of these insects to nourish their larvae.

Beltian bodies are a unique botanical characteristic of this mutualistic interaction, as they do not reflect any existing adaptations of the swollen-thorn acacia. Similar structures are absent in other legumes. An intriguing hint regarding the origin of Beltian bodies can be found in an undescribed acacia species located in eastern Costa Rica, which may serve as a prototype. Some plants of this species exhibit leaflets that feature a small, transparent apical segment. If one of the six *Pseudomyrmex* species residing in the occasionally large thorns of this acacia were to develop the ability to harvest these clear leaflet tips to enhance their diet, especially during the early dry season when food is limited, a new dynamic could begin to emerge (12). This potential food source would likely contribute to the growth of the ant colony, resulting in increased foraging behavior on the acacia's surface. This heightened activity could encourage greater production of leaves and flowering branches, further bolstering the ant population and promoting the development of more nutritionally beneficial structures. Over time, this process could culminate in the complete evolution of Beltian bodies. Moreover, the number and density of reproductive individuals within a *Pseudomyrmex* colony are closely tied to the colony's overall size, underscoring the advantages of developing such a food source [13]



Plate 5: Beltian bodies near leaflets on Acacia plant

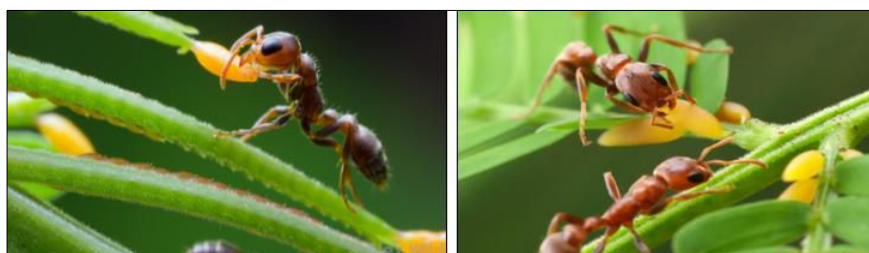


Plate 6: Acacia ant collecting Beltian bodies (Source: Naturalis Historia)

4.5. Each species with a few host-specific phytophagous insects, able to feed in the presence of ants

Most species of acacia host a limited number of insects that specifically target them, along with a larger, more varied group of more generalist feeders that may occasionally feed on them. Frequently, a primary feeder on one or two acacia species also acts as an occasional feeder on various other species within the subfamily Mimosoideae. These insects, in conjunction with rodents during the dry season, contribute significantly to the mortality rates of small, unoccupied swollen-thorn acacias. In the case of swollen-thorn acacias, the resident acacia-ants can be seen as a substitute for some of the physical and chemical defenses found in other acacia species. Furthermore, these ants serve multiple purposes, as

they actively work to eliminate a wide range of insect species.

Like their counterparts, swollen thorn acacias host a limited group of phytophagous insects that consume their foliage. These insects exhibit a range of morphological and behavioral characteristics that enable them to feed alongside the protective ants. Notable adaptations include an impenetrable cuticle that allows species such as *Pelidnota punctulata* to feed without showing any avoidance behavior towards the ants. Certain insects, like *Syssphinx mexicana*, are completely overlooked by the ants, allowing them to feed freely. Some species, including *Coxina hadenoides*, possess the ability to effectively ward off attacking ants during their feeding activities.



Plate 7: (A) *Pelidnota punctulata*, (B) *Syssphinx Mexicana*, (C) *Coxina kadenoides* (Source: Wikipedia)

It is likely that the early swollen-thorn acacia supported a variety of insects feeding on it. As the interaction between ants and acacias evolved over time, many of these insects were probably displaced from the acacia. Nonetheless, this adaptation provided a substantial food source for any insect capable of developing a strategy to feed alongside the obligate acacia ants. This scenario has occurred in at least nine distinct insect species that are now exclusively associated with swollen-thorn acacias, including *Syssphinx mexicana*, *Pelidnota punctulata*, *Coxina kadenoides*, and *Rosema dentifera*. Additionally, there are two insect species that specifically target the small, naturally unoccupied populations of seedlings and suckers: A) *Mozena tomentosa* and B) *Aristotelia corallina*. These adaptations illustrate the remarkable strategies these insects employ to coexist with ant defenders while feeding on swollen thorn acacias^[4].

Herbivores that primarily consume plants protected by ants have developed specialized tactics to evade attacks from ant workers. When a plant interacts with various ant species, these herbivores may either customize their avoidance strategies for individual ant species or utilize adaptable methods that work effectively across different ant-plant

relationships. Coronado-Rivera *et al.* (2020)^[5] investigated this interaction by studying the behavior of herbivores on Acacia trees defended by ants. Their research aimed to determine whether the herbivore *Piezogaster reclusus* showed a preference for certain ant species and how its evasion strategies varied with different ant species present on the Acacia tree.

The findings indicated that *P. reclusus* was most closely associated with Acacia trees that hosted *Pseudomyrmex flavicornis* and *Pseudomyrmex spinicola*, while it had minimal association with trees occupied by *Pseudomyrmex nigrocinctus*. It was observed that these herbivores mainly frequented younger leaves and were less commonly found on older leaves or the trunk. Importantly, the evasive behaviors exhibited during their movements did not depend on the specific ant species associated with the insects, implying that, similar to herbivores that feed on chemically protected plants, those living on ant-defended trees may evolve distinct strategies to overcome the plants' defenses protected plants, those living on ant-defended trees may evolve distinct strategies to overcome the plants' defenses.



Plate 8: (A) *Mozena tomentosa*, (B) *Aristotelia corallina*

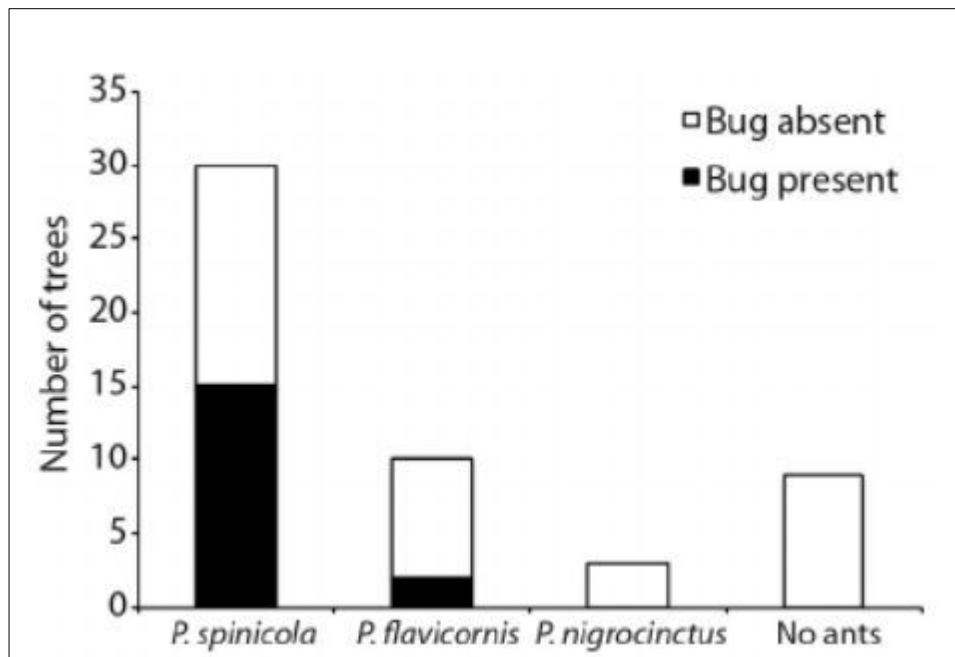


Fig 4: The number of *Vachellia collinsii* (Acacia) trees where *Piezogaster reclusus* bugs were either present (in black) or absent (in white) is determined by two factors: the presence of an ant colony on the tree and the species of mutualistic acacia ants inhabiting the tree.

4.6 Seeds dispersed by birds

Swollen-thorn acacias are distinctive among Central American acacias due to their bird-dispersed seeds, which are encased in a sweet, yellow pulp found in either indehiscent or dehiscent pods. The birds, such as the Black-headed Saltator (*Saltator atriceps*), Grayish Saltator (*Saltator coerulescens*), and Plain-tailed Brown Jay (*Psilorhinus mexicanus*), actively split open the pods to consume the sweet yellow pulp and, in the process, ingest some seeds. These birds typically drop the seeds in similar habitats to where they initially foraged. In contrast, the birds, including the Black-throated Oriole (*Icterus gularis*),

Derby Flycatcher (*Pitangus sulphuratus*), and Melodious Blackbird (*Dives dives*), open the pods to eat the surrounding pulp but discard the seeds, which often fall to the ground and may germinate near the parent tree^[17]. Ants generally attack the visiting birds, yet the birds are agile enough to avoid significant disturbance and continue feeding. Orioles, in particular, consume a large number of obligate acacia-ants along with the fruit but seldom eat the seeds. While bruchid beetles cause high seed mortality while pods remain on the trees, the birds' consumption of some seeds before the beetles can lay eggs helps ensure enough seeds survive to sustain the population^[23].

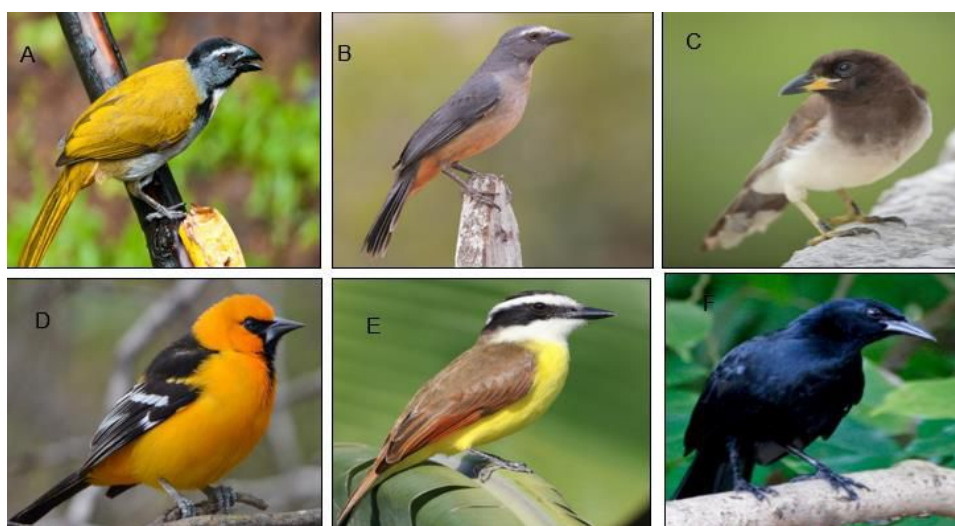


Plate 9: (A) Black-headed Saltator, (B) Grayish Saltator, (C) Plain-tailed Brown Jay, (D) Black-throated Oriole, (E) Derby Flycatcher, (F) Blackbird; Source: Wikipedia.

4.7 Bland tasting foliage

One distinctive feature of swollen-thorn acacias is their relatively mild flavor when compared to other Acacia species found in Central America. Although the specific compound that imparts bitterness to other acacias has not been identified, the absence of such bitterness in swollen-

thorn acacias indicates a lack of certain "secondary" plant compounds. This neutral chemical composition likely accounts for the heavy utilization of these unoccupied acacias by various insects that usually feed on different Acacia species. While these insects are typically specialized for other Acacia species, they readily consume the foliage of

unoccupied swollen-thorn acacias ^[12]. The lack of typical chemical deterrents makes swollen-thorn acacias more appealing to these insects, while still possessing enough general chemical characteristics to be recognized as suitable host plants.

It is proposed that the absence of these chemical deterrents in swollen-thorn acacias results from the presence of obligate acacia-ants, which effectively substitute for the lack of such compounds. This implies that these chemicals were initially developed as a defensive mechanism against insect herbivory rather than being mere incidental by-products ^[22]. Interestingly, the foliage of unoccupied swollen-thorn acacias is preferred by herbivores like cattle, brocket deer, and small rodents. Despite this preference, contemporary browsing vertebrates, with the exception of small rodents, which can inflict damage on many unoccupied shoots during the dry season, have a lesser impact on regrowth compared to insects in lowland tropical plant communities.

5. Characteristics of *Pseudomyrmex* associated with the coevolution of ant-acacia relationships.

5.1 Extremely quick and agile movers, highly aggressive

The *Pseudomyrmex* genus is distinguished by its swift and nimble worker ants. These ants typically detect intruders using their vision during the day and through contact around the clock. They can spot small moving insects from distances of up to four centimeters ^[16]. When attacking, the workers' rapid movements can sometimes cause them to miss their target and become distracted by other moving ants ^[11]. Upon contact, the ant will bite and sting the intruder, which usually prompts it to leave. If an ant is particularly agitated, it releases an alarm pheromone that heightens the activity of other workers, leading to a swift and concentrated response against the intruder. While they usually subdue only very small insects, which may be dismembered for larval nutrition, most of the remnants are often discarded from the tree. These ants also display similar aggression towards mammals, utilizing their barbed stings for anchorage. The sting of obligate acacia-ants is particularly more painful to humans compared to other *Pseudomyrmex* species ^[16].

5.2 Destruction of Living Vegetation

While non-plant-associated *Pseudomyrmex* species have not been observed attacking plants, obligate acacia-ants routinely bite and/or sting any foreign vegetation that makes contact with the canopy of swollen-thorn acacias. Seedling acacias are typically eliminated unless they possess swollen thorns and nectar-producing structures. Additionally, these ants actively destroy plants growing in a circular area beneath the swollen-thorn acacia ^[12]. Consequently, swollen thorn acacias tend to develop in a cylindrical space that remains largely devoid of foreign vegetation and the various effects it may cause, such as shading, mechanical obstruction, insect pathways, and potential fire fuel.

Thick clusters of swollen thorn acacias show a complete absence of other green plants. It is suggested that the response to foreign plants stems from the ants' inherent aggressiveness toward other insects and unusual objects in general ^[29]. While some nutrients may be derived from the sap of chewed plants, the following points argue against this being a primary motivation, such as the ants still attack even when there is an abundance of nectar and Beltian bodies available, all plant species are targeted; and worker ants

become aggressive toward plants as they age, typically beginning their aggressive behavior with insects first ^[12]. Thus, it is more likely that the selective pressure for heightened aggression toward plants functions similarly to the selection for aggression against animals by the obligate acacia-ants.

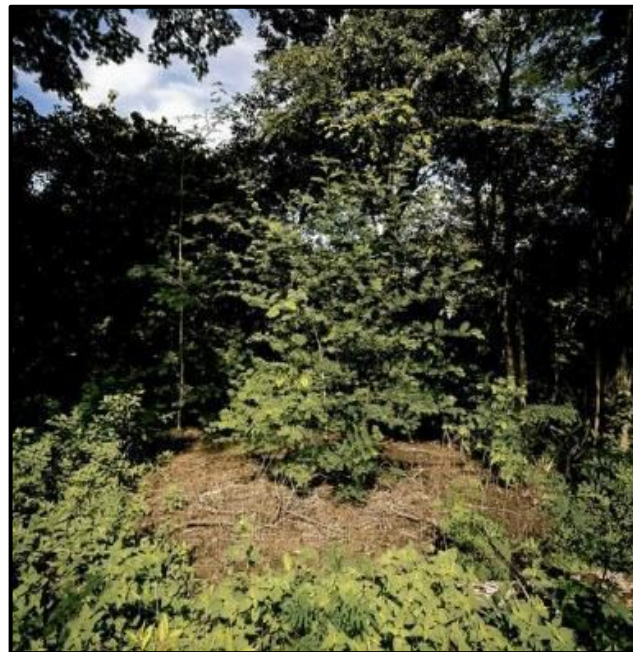


Plate 10: Destruction of surrounding vegetation

5.3 24-hour activity outside the nest

Obligate acacia-ants continuously patrol the surface of swollen-thorn acacias both day and night. In contrast, other *Pseudomyrmex* workers are primarily active outside the nest during the middle six to ten hours of daylight. The nocturnal activity of obligate acacia-ants is essential, as a significant amount of damage to unoccupied shoots occurs at night ^[16]. There are observable patterns in the density of these worker ants on the acacia's surface, with peaks aligned with periods of heightened insect activity, such as dusk and noon, as well as the times of maximum nectar flow, notably at dawn.

5.4 Steady Food Supply and Reliable Nesting Locations

Obligate acacia-ants receive reliable food and nesting sites from the acacia. Given the variable nature of food availability and intense competition for resources among other *Pseudomyrmex* and different ant species, an obligate acacia-ant colony would struggle to grow large enough to effectively patrol the acacia if it relied solely on foraging outside the acacia. Therefore, this interaction has necessitated a shift towards dependence on a plant-derived resource, Beltian bodies ^[3]. The primary adjustment involves the behavior of harvesting these bodies, a practice also seen in the genus *Solenopsis*, although without any association to this specific interaction. The consistent production of numerous new thorns each year to accommodate the growing ant colony is crucial to the overall system.

5.5 Founding Queens Scavenge for Food within Close Proximity

Once a founding queen of obligate acacia-ants identifies an unoccupied acacia and establishes herself within an empty thorn, she gains a reliable source of food. She only needs to

venture a few centimeters to access nectar for herself, as well as nectar and Beltian bodies for her larvae. In contrast, founding queens not associated with live plants must compete with other ants for food sources in the surrounding vegetation. The benefits of having quality nesting sites and readily available nectar likely played a significant role in increasing the density of *Pseudomyrmex* colonies on the original swollen-thorn acacias^[25].

Intense competition for the thorns on unoccupied acacia seedlings or suckers often occurs in areas disturbed by human activities. It is not uncommon for up to 100 queens to inhabit a single small seedling, leading to significant evasive maneuvers among them. Even if a queen secures a spot inside a thorn, she risks losing it, along with her brood, to another queen searching for food if she leaves. In regions with high production of queens from large, mature colonies, seedlings and suckers may experience long periods without successful new colony establishment. This phenomenon may act as a mechanism for population regulation, as an increased density of searching queens reduces both the rate of new colony formation and the survival of swollen-thorn acacia seedlings and suckers. Mortality rates among searching queens in these environments tend to be very high; this system likely reflects conditions from earlier periods when swollen-thorn acacias were more widely distributed in natural disturbance sites, necessitating a greater number of searching queens.

5.6 Multiple Queens per Colony and the Formation of Large Colonies

Most *Pseudomyrmex* species operate with a single egg-laying queen per colony, typically consisting of 100 to 2,000 workers when fully mature. Estimating the colony size for species residing in hollow branches presents challenges due to the subdivisions among branches. The queen remains in a single branch while eggs or first-instar larvae are transported to other parts of the colony. In colonies with a single queen, the workers do not permit the entry of new founding queens. This behavior promotes the continuous formation of new colonies instead of older colonies integrating the new queens, thereby preventing significant population fluctuations caused by the mortality of large colonies and ensuring the dispersal of colonies throughout the vegetation^[29].

Considering the challenges of food acquisition when not associated with living plants, it seems that a lone queen can generate enough larvae to utilize all the food gathered by the workers. Obligate acacia-ants maintain single-queen colonies consisting of 2,000 to 30,000 workers across at least four species. Despite plentiful food supplies, these colonies do not tend to grow substantially larger. In environments that strongly favor acacia reproduction, colonies may become overly dispersed across numerous shoots, which can hinder growth rates. In at least three species, colonies are known to accept new founding queens, leading to elevated egg production and the establishment of colonies with several million workers (comprising thousands of queens) spread over areas as large as half an acre of pure swollen-thorn acacia vegetation.

5.7 Reliant on Other Species Groups

Obligate acacia-ants rely entirely on swollen-thorn acacias for their survival in the wild. Although in a laboratory setting, glass tubes and honey can effectively replace thorns

and nectar, no alternative has been found for Beltian bodies. This dependency means that the fate of obligate acacia-ants is closely tied to the survival of the swollen-thorn acacias, provided that any catastrophic events occur so swiftly that these ants are unable to adapt outside of this mutualistic relationship^[30].

Interestingly, obligate acacia-ants are in a unique position to evolve traits that positively contribute to the survival and growth of swollen-thorn acacias. While similar cooperative or competitive dynamics likely exist in numerous interspecies interactions, they are often more difficult to demonstrate because most species maintain multiple interaction strategies with others. It is worth noting that the mutualistic relationship between obligate acacia-ants and swollen-thorn acacias serves multiple purposes, impacting their interactions with various other species in the ecosystem. Consequently, this relationship presents substantial opportunities to influence the outcomes of these interactions. Additionally, a feedback mechanism exists wherein the presence of a sufficiently mature ant colony capable of producing reproductives indicates the presence of a corresponding mature swollen-thorn acacia, which is likely to be capable of producing seeds or suckers. Conversely, a mature swollen-thorn acacia that can generate seeds or suckers robustly suggests that there is a well-established ant colony large enough to produce reproductives.

6. Ant-Acacia Symbiosis

The fundamental relationship between *V. cornigera* and *P. ferruginea* in the lowland regions of eastern Mexico exemplifies a fully evolved interdependent interaction.

- The queen ant locates an unoccupied seedling or sucker shoot of the acacia by navigating through the vegetation, both by flying and running. In this context, "unoccupied" denotes the lack of obligate acacia-ant workers outside the swollen thorns^[1]. Upon finding a suitable site, she either creates her own entrance hole in a green swollen thorn or utilizes a hole made by a prior worker or queen.
- She deposits her eggs inside the thorn and ventures outside to gather nectar from the foliar nectaries for herself and her larvae, as well as solid food in the form of Beltian bodies for the offspring.
- Colony expansion: As the colony develops, it begins to occupy all the thorns of the acacia, with typically up to 25 percent of the workers active outside the thorns at any given moment. At this stage, the queen's abdomen becomes significantly enlarged (physogastric), while the workers take on all roles related to labor, foraging, and defense within the colony^[27]. Approximately nine months of growth is required to generate enough mature workers capable of effectively and aggressively patrolling the exterior of the acacia. Once the colony reaches around 1,200 workers, production of the first winged reproductives commences, and thereafter, the generation of alates continues consistently, barring extreme starvation conditions. Over a span of three years, colonies can grow to contain as many as 16,000 workers, or even 30,000 after an extended duration. Throughout this period of expansion, the colony often relocates to nearby acacias (auxiliary shoots) within a distance of three to ten meters, although the queen typically remains in the original shoot (queen shoot)^[25].

- d) Protective duties of the workers commence: In colonies with more than 50 to 150 workers, the ants engage in continuous patrols around the outer shoot, day and night^[3]. Generally, they aggressively confront any insects on the acacia, successfully repelling them through biting and stinging, except for a few notable exceptions, namely insects that have developed adaptations to feed in the presence of ants. Furthermore, the workers will attack any foreign plants that make contact with the foliage of the swollen-thorn acacia or grow within a radius of 10 to 150 centimeters beneath the acacia, ensuring that the plant has a cylindrical area free from other vegetation.

Experimental evidence suggests that the swollen-thorn acacia has lost, likely due to evolutionary processes, its capacity to endure damage from herbivorous insects and competition from neighboring plants without the protective presence of obligate acacia-ants. To investigate the dependency of *V. cornigera* on *P. ferruginea* in lowland eastern Mexico, researchers experimentally removed ants from the acacias by either clipping thorns or cutting and burning shoots containing the ants, subsequently observing the growth of unoccupied stumps. In the experimental subplots, the shoots and new suckers grew without the presence of ant colonies for seven to nine months, and their development was compared with that of occupied shoots in control plots^[13]. The findings from these experiments indicate that if the obligate acacia-ants were suddenly wiped out, the acacia population would face a significant risk of decline, possibly leading to extinction.



Plate 11: Acacia ant cutting entrance hole on domatia

7. Discussion

7.1 What is domatia?

Ans. Domatia are specialised structures (hollowed thorn in case of bull horn acacia) found on some plant species that provide shelter and nesting for ants. In ant-plant interactions, the ant that inhabit domatia provide plants with various benefits, such as protection against herbivores, pathogen control, and soil nutrient cycling. In return, the plant provides the ants with shelter, food, and other resources.

7.2 Is the tree *Vachellia cornigera* present in India?

Ans. No, *Vachellia cornigera* is not present in India. It is a species of tree that is native to Central America and Mexico and is commonly known as the "bullhorn acacia" due to the distinctive shape of its thorns.

7.3 Is the ant *Pseudomyrmex ferrugineus* present in India?

Ans. No, *Pseudomyrmex ferrugineus* is not present in India. It is a species of ant that is native to the Americas, particularly in tropical and subtropical regions of Central and South America.

7.4 How many *Pseudomyrmex spp.* ant colonies inhabit a single bull horn acacia tree?

Ans. The number of ant colonies living in one bullhorn acacia tree can vary depending on the size of the tree and other environmental factors. Some large trees can support multiple colonies of ants, while smaller trees may support one colony. Studies have reported anywhere from one to six *Pseudomyrmex ferrugineus* colonies living in a single bullhorn acacia tree. However, the average number of colonies is usually around two or three.

7.5 Are there any ant-plant interactions being studied in India?

Ans. Ant-plant interactions are diverse and widespread in India, with many species of ants and plants involved in various types of associations. One of the most extensively studied ant-plant interactions in India is the association between the ant species *Crematogaster rogenhoferi* and the plant species *Macaranga peltata*. This plant provides food and shelter to the ant colony, while the ants defend the plant from herbivores and competitors. Another well-studied ant-plant interaction in India is the association between the ant species *Camponotus compressus* and the plant species *Humboldtia brunonis*. This plant provides nectar and housing to the ants, while the ants protect the plant from herbivores. Other notable ant-plant interactions that have been studied in India include the association between the ant species *Tetraponera rufonigra* and the plant species *Acacia chundra*, and the association between the ant species *Crematogaster* species and the plant species *Hirtella racemosa*.

8. Conclusion

Bull horn acacia and the ant species *Pseudomyrmex* thus offer an understanding of quite specific mutual relationship synergizing the evolution process. The typical characters of acacia such as production of beltian bodies, EFN, domatia favours dependency. Similarly, the action of ants leading to exploitation of these rewards and offering protection in return has led to evolution of such unique characters in acacia. Thus, glancing through ants and bullhorn *Acacia mutualism* explains the important process of evolution wherein interacting partners in a mutualistic relationship coevolve by reciprocally influencing each other's evolution. This mutualism and coevolution are a response towards survival, where both invest their energies accordingly based on the requirement. These behavioural responses can be exploited for action toward habitat protection. Many such evolutions are crucial for the surrounding living organisms making the whole ecosystem balanced.

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