The Herbivore's Dilemma: Never Enough Nitrogen

NAOMI E. PIERCE and ANDREW BERRY

One of the stranger features of the natural world is that a crucial component of living systems manages to be ubiquitous and hugely abundant while at the same time inaccessible and, for many species, hugely difficult to obtain. Nitrogen (N) is a key chemical building block in many of the molecules at the heart of life—it is required for nucleic acids (DNA and RNA) and for proteins—meaning that getting enough N is inevitably a major preoccupation of every living species. Approximately 80% of the atmosphere consists of nitrogen gas, so surely nitrogen should never be in short supply. Maybe there are just a few species living in obscure, airless environments that suffer from chronic nitrogen shortages? No, it turns out that the vast atmospheric reserves of nitrogen gas cannot in fact be harnessed by most species, because the N_2 gas molecule is so chemically inert. Nitrogen is typically only biologically useful when it is in a modified chemical form. Bizarrely, then, many species find themselves to be the metabolic equivalents of a thirsty sailor adrift in a small boat in the middle of the ocean, mocked by an overwhelming and tantalizing vision of an endless but utterly inaccessible supply of the missing resource.

A total of 25 chemical elements are known to be essential for life, but most of these—for example, potassium, calcium, and sulfur—are required in relatively small amounts. Just four elements collectively represent some 96% of living matter: C, H, O, and, yes, N. Imagine that you are a plant. You have the ability to photosynthesize, meaning that you can combine atmospheric carbon dioxide with water to produce sugar—a single process that takes care of your carbon (C), oxygen (O), and hydrogen (H) needs. How, then, can you meet your N needs? With difficulty, is the short answer. One source of N for plants is through recycling: when an organism dies, it is chemically dismantled by soil-dwelling bacteria and fungi that act as decomposers, recycling the dead organism's molecules. In this way, N in the appropriate form is present in the soil and available for uptake through the plant's roots. Ultimately, however, recycling is insufficient. Because of the inefficiencies inherent to the process, there is net loss of N in each go-round of the cycle. How, then, does new N enter the system? There are two potential sources: that mighty atmospheric reservoir and/or all that N locked up in rocks. In fact, like atmospheric N, the latter is unavail-

able to plants because the breakdown products of eroding, dissolving rock do not yield plant-friendly forms of N. So, we are stuck with the atmosphere: new N input into the system must come from the atmosphere. There are two main routes. One is basically serendipitous: about 5% of the usable N entering ecosystems comes from N that is dissolved by rain or through the accumulation of tiny particles (effectively N-rich dust). The other is the product of one of nature's more remarkable partnerships. Plants themselves may not have cracked the chemical problem of converting atmospheric N into usable N—a process known as nitrogen fixation—but microbes have. Some plants, most notably legumes, host on-board platoons of these N-fixing bacteria. The bacteria have a home and access to the nutrients they need, paying, as their part of deal, rent to the host plant in the form of usable N.

For plants, it should be clear: N is a headache. Plants have accordingly evolved to minimize their dependence on N. There is, of course, no getting away from those fundamental N requirements, for nucleic acids and proteins, but plants have evolved to make maximum possible use of their abundant photosynthesis-derived C, H, and O resources. Many structural components of an animal's body are made of protein, but plants have developed a carbohydrate-based architecture (carbohydrates are made of C, H, and O). Cellulose, which forms the cell wall of every plant cell, is a carbohydrate. Plants have in part solved their N problem by downplaying its significance in their biology. When you are looking at an animal, you are essentially looking at protein; when you are looking at a plant, you are looking at carbohydrate.

Plants transmit their N problem up the food chain. Animals, regardless of where they sit in the food chain, have bodies with roughly comparable chemical compositions. A herbivore—a rabbit, say—is, from a purely biochemical point of view, similar to a predator—a fox. This makes life hard for the rabbit, which is eating N-poor plant material but nevertheless has high N (i.e., protein) requirements. Contrast the rabbit's blighted situation with that of the fox, which eats the rabbit: there is an excellent correspondence between the fox's nutritional requirements and the composition of its food, whereas the rabbit is forever struggling to extract enough N from its N-poor plant food in order to compensate for the mismatch between the plant's composition and the rabbit's nutritional requirements. The herbivore's dilemma, then, is how to convert a low-N diet into a high-N lifestyle.

∼

For biologists who study herbivores, this never-ending N shortage is an ever present component of the ecological and dietary equation. Here we describe three case studies—each one a completely different approach to studying natural systems—in which the bottom line is the same: that N really, really matters.

∼

The typical herbivore strategy for overcoming dietary N deficiencies is to eat a lot. Think of cattle, forever chewing away; compare this with a pride of lions, spending most of the day lounging around and only occasionally heading off to snag a gazelle. All sorts of adaptations have arisen to facilitate this high through-put herbivore strategy, but none more striking than what we see in Lepidoptera, the butterflies and moths. Here the two fundamental processes of living things, eating/growth and sex/reproduction, have been segregated into distinct and specialized life stages: the caterpillar, which is basically a food-processing machine, and the adult, the butterfly or moth, which is basically a mobile set of genitalia (and, in the case of females, a dedicated egg distributor). Of course, there is more to a caterpillar's life than just eating: for example, predator evasion has to be another high priority, because there is no point in being the best converter of plant matter into body mass on the planet if you are then picked off by a roving bird. Caterpillars have evolved a remarkable range of defensive strategies, including being cryptic, sequestering in their bodies plant poisons (so that the caterpillar itself is poisonous), and constructing a feeding "shelter" such as a leaf roll. One exceedingly clever defensive strategy is that employed by the caterpillars of butterflies belonging to a large and diverse group, the Lycaenidae, consisting of the blues, coppers, and hairstreaks. Caterpillars of many of these species recruit ant guards to defend them against predators. The essence of the relationship is simple: the caterpillars have specialized glands that produce substances that both appease ants that might otherwise be threatening predators themselves, and also reward them in return for defense against their enemies.

A striking example of this ant–caterpillar relationship occurs between an Australian lycaenid, Ogyris genoveva, and its attendant ants, several species in the genus Camponotus. The caterpillars feed on mistletoes that parasitize eucalyptus trees, but you will not find the caterpillars if you go searching for them on the food plant high in the tree during the day; rather, you will find them in carefully constructed galleries in the soil at the base of the tree. The ants have built these galleries specifically to house the caterpillars, and it is here that the ants look after their charges during the day when it is unsafe to feed exposed on the leaves. Come evening, the ants, acting just like cattle dogs, herd their caterpillar cows up the tree trunk, and out on to the food plant. The caterpillars feed all night, all the while tended by ants, before being shepherded back down the tree to their earthen byres at the base at dawn. The relationship is fully agricultural: just as a farmer looks after his cattle in return for milk, so the ants tend the caterpillars in return for their secretions. Remove the ants, and the caterpillars are vulnerable to predators and parasites of all kinds.

Another Australian species, the imperial blue butterfly, Jalmenus evagoras, has become the workhorse of lycaenid ant biological research because it is rela-

F i g u r e 1 Ant–caterpillar relationship between an Australian lycaenid, Ogyris genoveva, and its attendant ant (genus Camponotus).

tively easy to work with experimentally and because its populations are easily located. The relationship between J. evagoras and its attendant ants (a handful of species of Iridomyrmex) is not as baroque as that between O. genoveva and Camponotus, but it nevertheless features the key hallmarks of lycaenid–ant interactions:

- The caterpillars are heavily tended by ants and produce an amino acid-rich secretion that is highly prized by the ants. Ants actively solicit the substance from the caterpillars, stimulating the specialized glands on the surface of the caterpillar with their antennae. Since amino acids are the building blocks of proteins, the caterpillars are sacrificing their precious N to reward their ant guard. Ant-associated lycaenid caterpillars are thus in a double bind: they are as subject to the herbivore's N shortage as any other caterpillar, but they have an additional N requirement—to recruit and maintain their ant guard.

- Female butterflies accordingly have two priorities when looking for sites to lay their eggs: they seek out appropriate host plants (various species of Acacia) as well as appropriate tending ant species. Because the presence of J. eva-

goras larvae on a tree attracts ants, females also use the presence of other caterpillars on a tree as a cue in making their egg-laying decisions.

- Caterpillars are dependent upon their ant guard for survival. It is easy to carry out simple field experiments in which ants are excluded (a sticky barrier around the base of the tree is enough to prevent ants from gaining access to the upper parts of the tree and the caterpillars feeding there) and the results are unambiguous: if deprived of attendant ants, the probability of caterpillars surviving to the adult stage in the wild, exposed to predators, is negligible.

- Ants are especially effective at repelling parasitoids, both specialized wasp and fly species. Given the chance, these parasitoids will lay their eggs in or on a caterpillar and the resulting larva will then set about eating the caterpillar from within, ultimately killing its host.

- We know that the metabolic cost to a caterpillar of maintaining an ant guard is substantial. If we raise J. evagoras caterpillars without ants, they produce bigger adults. Note that this experiment cannot be done in the field because in the absence of ants, caterpillar populations are devastated by predators; rather we have to bring the food plants and larvae into a predator-free environment such as a greenhouse or some other enclosure from which predators are excluded. Size is a significant issue for these butterflies because larger males are more successful in fights among malesfor mating access to females that are a common feature of this species. It is important, too, for a female to be large because size is a strong predictor of the total number of eggs she can produce in her lifetime.

Why are lycaenid caterpillar secretions so attractive to ants? Not many lycaenid species' secretions have been chemically analyzed, but it would appear that secretions vary considerably according to the specifics of the lycaenid–ant relationship. Species whose caterpillars are only occasionally associated with ants, such as the Provence chalk hill blue, Lysandra hispana, typically secrete mostly sugars with only traces of amino acids. However, species with stronger ant associations have been recorded to produce secretions that are rich in amino acids, with one or a few amino acids particularly prominent in their secretions. For example, one parasitic species, Niphanda fusca, whose caterpillars are taken into the ant nest where they are fed mouth-to-mouth like cuckoos by their host ants, secrete sugars plus high concentrations of the amino acid glycine. Caterpillars of Jalmenus evagoras secrete sugars plus high concentrations of the amino acid serine. The Japanese scientists who first noticed this pattern of amino acid

coupled with sugars suggested that this combination may act like an "umami" substance, a little like monosodium glutamate in Chinese food—ants find the combination of the amino acid and sugars much more attractive than either component alone.

Perhaps not surprisingly, lycaenid biology is even more N-dominated than that of other "normal" herbivores. It seems, for example, that the N requirement has profoundly shaped the evolution of host plant choice in this group of butterflies. Lycaenids whose caterpillars associate with ants feed disproportionately on legumes compared to species whose caterpillars are not tended by ants. Because of their association with N-fixing bacteria, legumes are typically richer and more constant over time in N than most plants. Similarly, lycaenid caterpillars have a predilection for relatively N-rich parts of plants, preferring, say, flowers, seed pods, and young terminal foliage to mature leaves.

As any gardener knows, it is simple enough to enhance plant growth rates by adding fertilizer to the soil. While fertilizers are typically complex chemical supplements, a critical ingredient involves some form of plant-accessible N. The gardener is facilitating plant growth by eliminating the I-need-more-N constraint.

Could using fertilizer to manipulate levels of N available to caterpillar host plants affect the interaction between the caterpillar and its ant attendants? Would caterpillars fed on a more N-rich diet be better at recruiting an ant guard? The experiment was simple: seedlings of the host plants were either treated with fertilizer or not, and the impact of enhanced N–availability traced through the plant–lycaenid–ant system. Jalmenus evagoras feeds on several species of Acacia, which is a legume, meaning that it has root nodules containing N-fixing bacteria. This is, as we have seen, presumably one of the reasons why it is attractive as a host plant for lycaenid species in the first place. Because of its N-fixing bacteria, Acacia, like other legumes, is less N-stressed than other non-N-fixing plants. Regardless, these Acacia species can nevertheless be N-limited themselves, meaning that they respond well to fertilization. Abundant N in the soil enhances growth and results in more N-rich foliage in these plants.

The first finding confirmed the experiment's premise that fertilization affected host plant quality. The plants in the group raised on water alone had lower leaf N content than the plants in the group raised on water with a liquid fertilizer mixed in. Now came the interesting part: would this difference in host plant quality have an impact on J. evagoras caterpillars and their attendant ants?

This answer was a resounding yes: not only were the caterpillars on fertilized plants tended by more ants than their counterparts on plants that had not been treated with fertilizer, but, critically, they were more likely to survive. Caterpillars eating a more N-rich diet were able to attract and maintain a larger ant guard than caterpillars on a relatively N-poor diet, and increasing the size

of its ant bodyguard duly enhanced a caterpillar's chances of survival. It also increased the attractiveness of these plants to females laying eggs. When a female lands on a plant, she can taste its quality with receptors in her feet and use this information to decide whether or not to lay an egg. Females laid many more eggs on fertilized plants than on unfertilized ones.

One possibility for the increased survival of caterpillars on fertilized trees was simply that their diet was better. Maybe, then, the survivorship effect was not actually related to ant bodyguard amplification, but, rather, to better nutrition. The control for this was to run the same experiment in the absence of ants. Remember, though, that ants are essential to the survival of J. evagoras, so it is impossible to assess the impact of nutrition on caterpillar survival without ants in the wild, simply because the caterpillars in the study will be promptly exterminated by predators. Therefore, the control involved re-running the experiment in the same location as the with-ants/predators version, but in a bush house constructed primarily from mosquito netting. In the absence of ants and predators, did the caterpillars survive better on relatively N-rich foliage? No. There was no difference in caterpillar mortality between fertilized and unfertilized trees, suggesting that, in the absence of ants, the caterpillars are gaining sufficient N for their growth and survival needs from the relatively low-N foliage. The extra N available to caterpillars on the fertilized plants is—in the absence of ants—surplus to requirement. Because we can now be confident that it is not the N content of the leaves per se that gives caterpillars on fertilized plants a survival advantage, we can conclude that the survival difference between caterpillars on fertilized versus unfertilized trees is attributable to the former's ability to attract a larger ant bodyguard.

How do caterpillars on fertilized plants manage to attract more ants? Is the secretion they produce more N-rich (and therefore more attractive to the ants)? Or are they simply able to produce more of it? These remaining questions are for future experiments in which the chemical composition and the volume of the secretions will be monitored. What, however, is clear is that N is the key player in this story. Jalmenus evagoras caterpillars, as herbivores, are inevitably N-stressed, but the added strain on their limited N resources of producing an N-rich incentive for their ant bodyguards means that they there is indeed never enough N. It is not surprising, then, that we see N availability as a major player in the evolution of these butterflies: recall that they tend to be limited in their host plant choices to plants that are relatively N-rich. The remarkable finding from this set of experiments is the long reach of N. It was introduced at the base of the food chain—as a raw nutrient to be taken up the fertilized plants' roots—and it worked its way up through the system to the extent that it acted as a determinant of the size (and therefore efficacy) of a caterpillar's ant bodyguard.

～

F i g u r e 2 Adult *Jalmenus evagoras*.

Another approach to studying the role of N in caterpillar–ant interactions is to reconstruct the butterflies' phylogeny—their family tree. With a phylogeny in hand, we can ask questions about specific evolutionary events: whether, for example, ant association in a specific species or group of species evolved de novo or was inherited from ant-associated ancestors. In essence, we are asking here whether or not the focal group's closest relatives are also ant-associated, which is why we need phylogenetic information telling us who or what are the focal group's relatives.

The premise of molecular phylogenetics is simple: the DNA sequences of a particular gene in two closely related species are similar because they share a relatively recent common ancestor, meaning that there has been a short time (by evolutionary standards) for the two sequences to diverge as they accumulate mutations through time. When we look at two distantly related species that have a relatively ancient common ancestor—more time for mutational differences to accumulate in those DNA sequences along each lineage—we find the DNA sequences to be significantly more divergent. The process of generating a phylogeny involves getting molecular sequence data from the species you are interested in. This, then, is fed into a computer algorithm which reconstructs

the tree on the basis of the "similar sequence = close relative; different sequence = distant relative" premise.

The family tree of lycaenid butterflies has proved a rich source of insight into the evolution of ant–butterfly relationships, but one finding in particular is strikingly relevant to the caterpillars' N problems: carnivory has evolved repeatedly in lycaenid caterpillars. Carnivorous caterpillars? The very idea is oxymoronic: caterpillars are leaf-processing machines, the cows of the insect world, not the lions. As we have seen, the two-phase life history of butterflies and moths permits specialization, with a caterpillar being all about growth, and an adult being all about reproduction. Specifically, caterpillars are specialized as herbivores. Sluggish because their leaf diet does not run away from them, caterpillars are basically specialized, elongated, mobile plant-digesters. That such an inert grub should become a predator seems unlikely. Nevertheless, across butterflies and moths, "carnivory" (or, more technically speaking, "aphytophagy," meaning feeding on things other than plants, which can include feeding directly on the tissues of other animals, or their secretions or regurgitations) has evolved repeatedly. Within the Lycaenidae, we can find at least 13 occasions when an aphytophagous species appears to have evolved from ant-associated herbivores: each of the 13 carnivorous species has as its closest relatives ant-associated species that eat plants. Lycaenids represent only a small proportion of all butterflies and moths, and yet a relatively high percent of the origins of carnivory occurred within the group. Why are the lycaenids so prone to evolving carnivory? Two reasons: ants and N.

Because many lycaenids have close associations with ants, opportunities arise that result in the exploitation of the ants. Typical mutualistic ant–lycaenid interactions, in which the caterpillar trades an N-rich secretion for protection, involve considerable inter-specific communication. This is particularly true in cases where the relationship is highly specialized: a species of caterpillar interacts only with one or a few closely related species of ant. In order to recruit the right ant guards, these lycaenid species have apparently evolved chemical signals that are specific to their attendant ant species. Once the ants' chemical communication code has been cracked by the butterfly, the potential exists to use these communication skills to subvert a mutualistic relationship, turning it into a parasitic or carnivorous one. Take, for example, the extraordinary life history of the large blue butterfly, Maculinea arion. Caterpillars start off as typical lycaenid–ant mutualists, feeding on flowers and being occasionally tended by ants. When, however, a caterpillar reaches a certain point in its development, it changes its chemical signal to the ants, inducing them to carry it into the ant nest. There, a wolf in sheep's clothing, it sets about eating the ant larvae. As we have already seen with Niphanda fusca, another lycaenid species, this chemical trickery cab be so sophisticated that caterpillars can induce the ants

to feed them directly through regurgitation in the same way that the ants feed their own young. It is clear that the close relationship between lycaenids and ants has facilitated the evolution of predatory and/or parasitic behavior. This is reflected by the lycaenid phylogeny: predator/parasites typically evolve from mutualistic species.

The second reason for the remarkable tendency of the lycaenids to evolve carnivory relates to N. Because of ant association, lycaenids are typically even more N stressed than other herbivorous insects. What is the simplest way to increase the N titer in your diet? Become a meat-eater—suddenly all those N constraints are gone. It is no surprise, then, that one of the most N-challenged groups of Lepidoptera is the one that is particularly prone to evolving carnivory.

∼

There is no doubting that lycaenid biology (and evolution) is in part dictated by the demands of N. But what about the ants? The mere fact that ants are so enthusiastic about tending lycaenid caterpillars for N-rich rewards suggests that they, too, are severely N-limited. But how have ants gone about dealing with their N problems? One possibility is microbial: some insect groups that are dependent on nutrient-poor diets have evolved mutualistic relationships with microorganisms that inhabit their guts; in exchange for housing, the microbes provide metabolic assistance typically by breaking down hard-to-digest plant matter.

An extensive literature exists on the microorganisms—both bacteria and protozoa—that live in guts of termites, helping their termite hosts digest the unpromising woody material that is typically the main component of a termite's diet. However, less is known about the on-board microorganisms of ants. One group that has been well studied includes the carpenter ants, which harbor microbes that enhance the nutrition of their host ants. And one species of ant in the genus Tetraponera has been shown to have bacteria in its gut capable of fixing atmospheric nitrogen. But what about the presence or absence of bacterial symbionts throughout the entire family? How widespread are symbiotic gut bacteria?

The easiest way to identify what microorganisms are present in a sample (a drop of water, a pinch of topsoil, say, or the midgut of an ant) is with DNA sequences. Certain genes have been widely sequenced across microorganisms such that we now have diagnostic sequences for these genes for many species of microorganism. When you extract DNA from an ant for sequence analysis, you essentially squish the whole animal or the part of the ant you are interested in, such as the gut, and extract DNA from the resulting ant-mush. Obviously, there is a great deal of ant DNA in ant-mush, but ant-mush also contains DNA from

any microorganisms that might have been lurking in the ant's gut when the ant was collected.

A survey of 283 species from 18 of the 21 subfamilies of ants showed that most of them did indeed harbor many species of bacteria. Often there are specific groups of microbes that are found only in a group of closely related ant species, suggesting that these are not transient bacteria that just happened to be in the ant gut at the time when it was collected.

So, ants do indeed host a rich and diverse gut microorganism ecosystem. Perhaps most interesting, however, is the observation of a decidedly nonrandom element to the distribution of microorganisms across the ants. Specifically, it seems that a certain group of microbes, the Rhizobiales, tend only to be present in ants known to live primarily on largely plant-derived foods such as nectar from flowers and from specialized plant organs called extra-floral nectaries, and the sugary substance, honeydew, produced by aphids. Many of these ant species dwell primarily in forest canopies.

Appreciating the significance of this correlation requires some background information on both ants and Rhizobiales. Let's start with the ants. Ants are a remarkably dominant feature of almost all terrestrial ecosystems. By and large, they fulfill similar roles in different geographic locations: they are generalist scavengers-cum-predators. Other insects are on the alert when ants are on the march. Many ants have plant-derived material in their diet, especially nutritious (i.e., N-rich) plant components, such as seeds. Some ant species that have taken plant eating to an extreme are found in the forest canopy and appear to be almost exclusively "herbivorous" in the sense that they eat plant-derived food.

The assertion that canopy-dwelling ants are true herbivores is somewhat conjectural: although canopy-dwelling ants are widely assumed to be herbivorous, there is very little direct evidence to confirm this. And, because it is so difficult to study canopy-dwelling insects—the difficulty of getting into the canopy is prohibitive—a simple ecological solution—observing the ants foraging, for example—is impractical. Here recent technological advances in chemistry have come to the rescue. Chemical elements exist naturally in different forms—isotopes (the isotopes we tend to hear about are radioactive, but plenty of isotopes are not radioactive): carbon, for example, occurs naturally as C-12, C-13, and C-14, and nitrogen can be found in heavy (N-15) and light (N-14) forms. For complex chemical reasons relating to isotope-usage biases of different enzymes, tissue constructed from plant material differs in its N isotope make-up from tissue derived from a non-plant source, meaning that organisms can be classified as carnivorous or herbivorous on the basis of the ratio of heavy to light nitrogen ($^{15}N/^{14}N$) in their tissues. In short, there is a distinct isotopic signature in the chemical composition of an animal that declares it to be a her-

bivore or not. Ants have been broadly surveyed to determine whether they are carnivorous or herbivorous, and, yes, many canopy-dwelling ants fall solidly into the herbivore category.

It is clear that the ants involved in the ant-Rhizobiales association tend to be herbivores. Now, what of the Rhizobiales? Rhizobiales is an order of bacteria containing the key nitrogen-fixers in the root nodules of legumes. Recall that one of the few ways to inject atmospheric N into the ecological N cycle is through N fixation, and that it is only bacteria that have evolved the metabolic capability for doing this; the bacteria in question are Rhizobiales. These bacteria possess an enzyme called nitrogenase that converts atmospheric nitrogen to ammonia, which is typically then incorporated into the amino acid glutamate, thereby making the nitrogen metabolically available to the plant. Interestingly, this whole process is extremely sensitive to the presence of oxygen, so much so that many nitrogen-fixing organisms can only live in anaerobic conditions of low oxygen, as they do presumably in the root nodules of legumes or the specialized gut tissues of many herbivorous ants..

Looking across the phylogeny of ants, there have been five independent origins of herbivory, each one generating many descendant species. Members of all of these five groups possess Rhizobiales as gut symbionts, suggesting that the evolution of herbivory in ants is coupled in some way with the acquisition of on-board N-fixers. Is it just chance, then, that Rhizobiales are present in the guts of a particular subset of ants—those that are closest, among ants, to true herbivores? Surely not: herbivores are N-stressed, and Rhizobiales are N-fixing...QED!

The question immediately arises as to whether the Rhizobiales in the guts of herbivorous ants are indeed complementing their host ant's diet by fixing atmospheric nitrogen. The answer to this is maybe. Not all Rhizobiales have the ability to fix N, and some of the Rhizobiales found in the guts of herbivorous ants did not possess genes for nitrogen fixation. We can only assume that in these cases, the Rhizobiales enrich the diets of their host ants in other ways. If herbivorous ants are able to supplement their N supplies via on-board N-fixing bacteria, why do we not see other ants with on board N-fixers? After all, the Iridomyrmex ants tending J. evagoras caterpillars are willing to go to extremes for a N reward, suggesting that even ants that are not herbivorous are N-stressed. We do not know the answer to this, but it seems likely that there must be a cost to maintaining an on-board colony of Rhizobiales, such that the N-fixing strategy is only viable in instances of severe N stress, such as that experienced by herbivorous species.

∼

The N-centered findings of this study of ant microorganisms bring home once again just how limiting N availability is for many species. The herbivore's dilemma, then, is different from the omnivore's dilemma. Omnivores, by virtue of being metabolically capable of using a large range of foodstuffs, have to choose what to eat. Herbivores, constrained in their diet choice, are faced with a different problem: how, given the inherent deficiency of N in plant matter, do they make sure that they nevertheless get enough N? In the fertilized/unfertilized plant study, direct manipulation of levels of N in the food chain provided a way to measure how far up the food chain the ripple effect would resonate. The result showed that the downstream components of the system—the size of a caterpillar's ant guard and, therefore, its chances of survival—were incredibly sensitive to baseline manipulations. The N effect was big and far-reaching. The phylogenetic study revealed that, historically, lycaenid butterflies have been especially prone to taking the weird (for a caterpillar) evolutionary step from benign herbivore to ant parasite or predator. Here, the harsh dietary reality of N shortage has affected long-term patterns of evolution in the butterflies. The ant gut study was undertaken with no particular expectation of an N effect. It just fell out of the data, reminding us once again how absolutely central N acquisition concerns are to herbivore biology. In many ways, the third study was the most dramatic: we were searching for an N effect in the first one, and, to some extent, in the second, but not in the third. That the N issue cropped up so prominently even when we were not looking for it is the best possible illustration of the central and key role of N in herbivore biology.

SUGGESTED READINGS

Baylis, M. and N. E. Pierce (1991). The effect of host plant quality on the survival of larvae and oviposition behaviour of adults of an ant-tended lycaenid butterfly, Jalmenus evagoras. Ecol. Entomol. 16: 1-9.

Pierce, N.E. (1995). Predatory and parasitic Lepidoptera: carnivores living on plants. J. Lepid. Soc. 49: 412-453

Pierce, N.E. and D.R. Nash (1999). The Imperial Blue, Jalmenus evagoras (Lycaenidae), In: Kitching R., Sheermeyer, E., Jones, R. and N.E. Pierce (eds.). The Biology of Australian Butterflies (Monographs on Australian Lepidoptera Vol. 6); CSIRO Press, Sydney, pp. 277-316

Pierce, N.E., Braby, M.F., Heath, A., Lohman, D.J., Mathew, J., Rand, D.B., and M.A. Travassos. (2002). The ecology and evolution of interactions between lycaenid butterflies and ants. Ann. Rev. Entomol. 47: 733-71

Russell, J.A., Moreau, C.S., Goldman-Huertas, B., Fujiwara, M., Lohman, D.J. and N.E. Pierce. (2009). Bacterial gut bacteria are tightly linked with the evolution of herbivory in ants. PNAS 106: 21236-21241.