BEHAVIORAL ECOLOGY OF *NEOCHLAMISUS* LEAF BEETLES: THE ROLE OF FECAL CASES AND ASSOCIATED TRAITS

By

Christopher G. Brown

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Approved:

Professor Daniel J. Funk

Professor Dave McCauley

Professor Patrick Abbot

Professor Manuel Leal

Professor Jim Fordyce

This work is due to my parents, Glen and Sylvia Brown,
whose loving care brought me to the top of the hill

and

to my beloved wife, Jennell Talley,
whose patience and (mostly) gentle encouragement pushed me to the other side of it.

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CHAPTER I

INTRODUCTION

There's no place like home…

Many species of animals gather materials and attach and piece them together in particular ways to construct buildings. From protozoa to primates, animals across the kingdom are known to build cases, homes, traps, signals, and innumerable other kinds of animal architecture. Mike Hansell defines animal architecture as a behavior applied to materials to make a structure (Hansell 2005, 2007). The materials used are almost as diverse as the taxa that build with them and include mud, plant parts, silk, mucus, wax, paper, and even bubbles (von Frisch 1974, Hansell 1984, 2005). Some architectures are very intricate, e.g., weaverbirds delicately weave blades of grass together to form a cradle for their eggs (Collias and Collias 1984); others are amazingly durable, e.g., some potter wasp nests are thought to remain on the cliff face for thousands of years (Hansell 2007); others are quite large, e.g., 30 m “mima prairies” in South Africa built by termites and mole rats (Lovegrove 1993). Beavers offer a well-known and illustrative example of the intricacy, efficiency, and art of animal architecture. Beavers build dams out of sticks and mud to stop the flow of streams, which in turn create artificial ponds (Zurowski 1992). These ponds provide the beaver with ample food supply (bark) that is protected and easily accessible (underwater) in places where the surface freezes. Beavers also build an intricate lodge whose inner chamber is above the water's surface, but whose entrance is below, thus protecting the inside from terrestrial predators. The lodge is tightly constructed on most sides and is therefore waterproof and maintains warmth, but has loose construction on top for airflow, a perfect home for raising a family.

A given structure often allows its builder to cope with external conditions without major alterations to physiology or requiring many new genetic adaptations (Dawson et al. 1988, Danks 2002, Hansell 2005). For example, termites are physiologically sensitive to changes in humidity, yet some species often forage long distances from their nests across inhabitable terrain. They maintain optimal conditions by building long tunnels of mud, feces, and carton (i.e., chewed plant material), which allow them to forage away from home without distinct morphological characteristics to live in different conditions (von Frisch 1974). Many caddisfly larvae can be keyed out based on case structure when the larvae themselves are relatively indistinguishable (Stuart and Currie 2001). This might also be why insects (which have highly adaptable cuticles) are so morphologically diverse, whereas there are relatively few body types in spiders (which have highly adaptable web arrangements).

Materials are either collected, secreted, or both (Hansell 2005). Collected materials include, but are by no means limited to, sticks, leaves, mud, stones, shells, the building materials of other organisms, and other organisms themselves. This latter group is interesting because sometimes the material also benefits from the construction, as in sea anemones on hermit crab shells that benefit from the crab's mobility by filtering diverse areas for food. Other times they probably do not, as in leaves used by weaver ants (although living leaves apparently do a good job of removing carbon dioxide and adding oxygen to the tightly woven nest chamber [Gould and Gould 2007]). Secreted materials used in animal architecture include silk (spiders), saliva (swallows), wax (bees), “glue” (sticklebacks), and feces (see below). By secreting materials, animals can avoid any collecting costs incurred when gathering objects in the environment, but may suffer a cost of nutrients or energy when making the materials. Secreted materials, moreover, are consistent and uniform, although some processing is usually still needed, and allow the associated building behaviors to be simpler and more repetitive (Hansell 2005). Some materials are secreted for one purpose and collected for another. For example, down feathers, used to insulate adult ducks from weather and water, are also used to line nests (Hansell 2000). The palisade moth plucks out its own wing scales, used in flying and for coloration, to build a protective structure around its eggs (Gould and Gould 2007).

Although builders span most animal groups, the major taxa with constructing behaviors can be found in the arthropods and birds, and to a lesser extent the mammals (mostly rodents and humans). Despite their diversity and multiple evolutionary origins, Hansell (2005) divides animal-built structures into three main categories based on their function: food-gathering, communication, and shelter. I add locomotion to these categories, since it is a much different purpose, even though it is much rarer. Architectures are used to increase food availability in a number of ways, from traps to storage. Spiders and antlions are well-known trap makers, but another example would be caddisfly larvae that spin silk nets to filter the water current. One species of caddisfly builds a box with an intake tube and an outflow tube with a silk net spun across the middle (Hansell 2005). It sits tucked into a protected side chamber and collects food trapped in the net. In order to "sneak up on" prey, some insect predators cover themselves in debris from their habitats, such as leaf hairs or dead ants (Eisner et al. 1978, Brandt and Mahsberg 2002). These covers masquerade the predator as a common, less dangerous component of the environment: a veritable "wolf-in-sheep's clothing" (Eisner et al. 1978), thus allowing them closer access to food sources. Once built, food stores and some leaf-rolls and mines make food provisions easier to access and can increase food quality. *Callosobruchus maculatus,* a beetle that develops entirely in a single bean, builds walls within its chamber to reduce intraspecific competition so that multiple individuals can successfully emerge from one bean (Mano and Toquenaga 2008). And of course, many animals (mostly birds and primates) create tools, also considered animal architecture, which are used in food gathering and preparation (Hansell 2007).

Animal constructions are also used for communication, both between and within species. Male bowerbirds build intricate nest-like structures that are not used for rearing offspring, but rather serve as sexual signals by which females judge male quality (Diamond 1986). Male mole crickets of *Gryllotalpa australis* dig a chamber with two cones curving to the surface that amplify their mating songs (Daws et al. 1996). Some species of orb-weaver spiders add zig-zag style patterns to their webs (stabilimenta) that may serve as visual cues to lure prey or alert flying birds that might otherwise damage the web to their presence (Eberhard 1990).

Although fewer in number and not considered as a major category by Hansell (2005, 2007), some animal built structures are used for locomotion and dispersal. Individual caterpillars, mites, and spiderlings release silk strands, which catch the wind, and soar from one place to another, a method of movement called "ballooning" (Bell et al. 2005). Some caterpillars and spiders also release "draglines." These silken ropes slow their descent when falling and allow them to find their original location more easily.

More unusually, the violet snail *Janthina* floats on the water's surface by means of a raft of bubbles made by the snail's mucus (Hansell 1984).

By far the most common use of animal architecture is for shelter. Shelters provide a barrier between the builder and the immediate environment around it. Such homes include nests, burrows, mines, lodges, mounds, and condominiums. They serve to reduce predation by reducing detection (primary defense) and successful attack (secondary defense), and to reduce any harmful effects of abiotic conditions, such as temperature and humidity fluctuations (Hansell 1984, 2005, Danks 2002). Usually, shelters are built by parents for the protection of their young, and are not used by the adults after the breeding season. For instance, many birds and wasps build large shelters for eggs, but not winter shelters for themselves (Gould and Gould 2007). Sometimes though, especially in the holometabolous insects, juveniles build their own home. These range from relatively simple tunnels to intricate leaf rolls and shelters built by some caterpillars. Also common are larvae that build shelters around their bodies by sowing sticks, leaves, sand grains, and other objects together with silk to form a kind of tube or sack. These include the cases of caddisfly larvae (Trichoptera) and bags of the Psychidae (Lepidoptera). Not only do these structures provide protection from various environmental threats, they also allow the added bonus of motility, an unusual feature when considering most animal architectures.

Numerous studies have elucidated the adaptive benefit of animal-built constructions, but building behaviors can also incur costs, including energy loss and increased conspicuousness. Gathering or secreting, processing, and building a structure takes energy and nutrients that might otherwise be used for reaching a larger size or greater fecundity (Stevens et al. 1999, Otto 2000, 2000, Venner et al. 2003, McKie 2004). Some builders even construct a new nest every year. Alternatively, building may allow builders to spend less energy than other organisms, for example, by using traps to aid hunting. They may also be able to save more energy in shelters while resting than animals that must maintain stricter vigilance or regulate their own homeostasis. Another cost, however, is that builders are often more conspicuous than non-builders. Much architecture is large and immobile and thus can be easily found by predators and parasitoids, especially while in the act of building (Murakami 1999, Müller and Hilker 1999, Gould and Gould 2007). Since shelters often allow many conspecific individuals to live in a small confined place, such as bee hives and mole rat burrows, these species can also suffer higher rates of fungal, bacterial, and viral infection and more parasites than if they were living individually (e.g., Kopachena et al. 2007). Many birds (Hansell 2000) and mammals including badgers (Butler and Roper 1996), rats (Roper et al. 2002), and bats (Reckardt and Kerth 2007) avoid using the same roosting area for too long a time in order to avoid build-up of ectoparasites. This might be why some builders sacrifice the energy to build a new shelter every year. Despite these trade-offs, building behaviors have evolved numerous times across the animal kingdom.

In fact, animal architecture may even promote speciation in some instances (Gould and Gould 2007). Niche availability might expand with a change in building materials. Hansell (2007) tells a nice story of how architecture may increase diversity using a hypothetical wasp species that normally builds in clay. One population might begin adding plant materials, which allows building nests that are of lighter weight. Less heavy nests can be hung in trees rather than the previously required cliff face, thus increasing nesting opportunities and reducing competition and predation. Concordantly, new designs may evolve incrementally over time as populations are geographically separated. Building behaviors can be genetically simple (Dawson et al. 1988, Bult and Lynch 1997) and organisms can adapt to a different environments through changes in building behaviors without drastic changes in morphology. When genetic blueprints for separate building designs cannot be mixed effectively, there may be reproductive isolation via lower hybrid fitness, since hybrids do not build a functional structure (e.g., Dawson et al. 1988). This could eventually produce separate species. Populations of a building species can become specialized to use certain materials, such as leaves of a certain plant species when making a leaf-roll, and become less efficient on materials used by related populations. Social insects, in which multiple individuals contribute to one design, have to work together using the same "blueprint." Therefore, combining different genotypes reduces their ability to build an effective structure (Gould and Gould 2007, Hansell 2007). Reproductive isolation is especially likely when the structure is used as a mating signal, as in bowerbirds, or as a mating site, as in swallows, since females from different groups may choose males based on their building behaviors.

Architectures can change the environment, and thus selective pressures, for non-building organisms too, a type of niche construction (Hansell 1993). Beavers change the landscape dramatically (e.g., Rosell et al. 2005), but other such "ecosystem engineers" can be a little subtler. Old organ pipe wasp nests are used by cliff swallows to attach their nests (Hansell 2007). Cultivated fungi that are reared in an insect's structure can evolve in conjunction with their host insect (Farrell et al. 2001). Large structures often become home to a variety of other plants and animals. Several birds "squat" in termite nests and social spider webs (Brightsmith 2000, 2004, Kesler and Haig 2005) and may require unknown specializations to do so. Leaf shelters are known to induce dramatic changes in diversity within and around a host plant by attracting, and increasing niches for, other species (Ohgushi 2005). Therefore animal-built structures can greatly impact the morphology, behaviors, and ecology of the builder and other non-related organisms. Architectures too, uniquely supply quantifiable physical evidence of behaviors that persists across species bifurcations and geological time, thus allowing us to map many subtle behavioral traits in phylogenetic studies that would otherwise be lost to time (e.g., Winkler and Sheldon 1993).

It's a fecal matter…

One of the most intriguing building media, at least by human standards, is feces. Yet many organisms (mostly insects) have evolved amazing ways to deal effectively with their wastes (Weiss 2006). Feces in insects are used as an aggregation attractant (Suzuki 1985, Weaver et al. 1989), a way to find mates (Fettköther et al. 2000), or the way back home (Miller and Koehler 2000). Feces may also reduce competition and often act as an oviposition deterrent (Anderson et al. 1993, Anbutsu and Togashi 2002, Borg-Karlson et al. 2006, Addesso 2007). Leaf-cutter ants and fungivorous termites use it as fertilizer in their fungus gardens (Hansell 2005). Waste products are also the main link in aphid-ant mutualisms (Stadler and Dixon 2005). Fecal material may contain toxins that reduce predation. For instance, *Eloria noyesi* moths excrete an unusual feeding-deterrent known as cocaine when feeding on *Erythroxylum coca* (Blum et al. 1981). And other insects use waste products as a building material, as seen in thrips, termites, spittlebugs, lepidopterans, and beetles (Weiss 2006).

Most animals simply discard their waste products. It is often considered unhealthy to remain around fecal material since it can be infected with fungi and bacteria and may aid the transmission of parasites whose cycles include internal and external stages (Collison et al. 2004). However, Weiss (2003) found that keeping frass around did not affect survival and says that no studies have found a negative effect of living with frass, at least in insects (Weiss 2006). In fact, some species are known to incorporate antimicrobial properties in their excrement (Müller and Hilker 2004). Leaf-cutter ants are known to add specific antifungal properties in the "manure" used in their fungal gardens to prevent unwanted fungus from growing, but without affecting the desired "crop" species (Poulsen et al. 2005). Some shelter-dwelling insects whose frass accumulates in their shelters (e.g., tent caterpillars and some leaf-rollers) may emit antifungal chemicals to prevent pathogen growth (Weiss 2006). Perhaps insects are physiologically less likely to suffer from the negative issues of feces and this may explain why building with waste products is more prevalent in arthropods than vertebrates. This tendency may be even more likely in phytophagous insects. Excrement from a leaf-feeder may simply be less likely to become infectious than a meat-eater's, because of the chemical nature of their food source or differences in internal parasite quantity or life cycles. Phytophagous insects often sequester toxins and antimicrobial chemicals from their host plants and naturally such substances can easily be incorporated into feces. Insects, however, do face the danger of attack by predators and parasitoids, which can use feces as a kairomone to locate their hosts (Müller and Hilker 1999, Schaffner and Müller 2001, Weiss 2003).

As a building material, feces share the beneficial attributes of other secreted materials, mainly reduced collecting costs, uniform building blocks, and malleability. However, feces have one major advantage in that they are produced by almost all organisms as waste material. Unless the builder adds chemicals or other objects to the feces, there is no additional cost to the organism's metabolism (e.g., Olmstead and Denno 1992). Fecal material can be easily made of materials of different strengths and densities, such as digested and undigested plant material (Chaboo et al. 2008, Chapter V). Such composite materials (like reinforced concrete) are often better at resisting breaking under tension and compression (Hansell 2000). Feces may also exhibit the same thixotropic properties as mud, a material used by many builders. Using feces as a building medium is also beneficial since it instantaneously camouflages. It is a very earthy, natural color and blends in well with most habitats. The puriri moth caterpillar *Aenetus virescens* covers its tunnel entrance with silk and feces, making it barely distinguishable from the surrounding tree trunk (Buffalo Museum of Science website). Of course, fecal coverings also let a builder masquerade as feces. Even parasitoids that use feces to locate their host, may not expect to find their host under the feces itself.

The production of feces as a building material is dual-purpose. One is digestive, i.e., to acquire as many nutrients as possible and remove wastes effectively. The other is for building, i.e., to make a consistent building material that will hold together and serve whatever functions are intended (e.g., maintain humidity, prevent predation, etc). These requirements could select for specific types of feeding or differences in digestion and may induce tradeoffs. That is, the most nutritive food may not be the best for making buildings and thus a healthy individual may sacrifice structural integrity. Alternatively, producing feces that can best be used as a building material may enable less nutrient absorption, and thus more time feeding.

A special case…

As in several categories, the most prevalent users of feces might be the beetles. Tenebrionids use it as an aggregation attractant (Suzuki 1985, Weaver et al. 1989), weevils (Addesso et al. 2007) and long-horned beetles (Anbutsu and Togashi 2002) use it as an oviposition deterrent, and, as stated above, a seed beetle uses feces to build a wall to prevent competition within a bean (Mano and Toquenaga 2008). Feces in most beetle architectures are used for protection (Gross 1993, Weiss 2006). Cerambycid larval tunnels often get packed with feces and sometimes the larva is surrounded by it when pupating (Rodd 1951). Many create a thick "frass plug" that seals off the pupal chamber, which could prevent other organisms from accessing them (although the parasitoid *Megalyra fasciipennis* locates these plugs and oviposits through them [Rodd 1951]). A fecal covering is similarly used to protect ladybird beetle eggs (Arakaki 1988).

Feces used as shelter may be most common in the leaf beetles (Chrysomelidae) (Olmstead 1994, Müller and Hilker 2004). An estimated twenty percent of leaf beetle species (~7,000) cover their eggs or larvae in waste products (Vencl and Morton 1999, Vencl et al. 1999). These fecal coverings take a variety of shapes and sizes and serve to protect young from predators (Gross 1993, Weiss 2006) or desiccation (as suggested by Karren 1964, Damman and Cappucino 1991, Olmstead 1994, Nahrung and Marohasy 1997). Species of both Donaciinae and Criocerinae subfamilies are known to deposit substances, such as “glue” or "slime", on egg batches, which may attach the eggs to the substrate, protect them from parasitoids, or contain beneficial symbionts (Schmitt 1988, Müller and Hilker 2004). Almost every other subfamily within Chrysomelidae contains species that are known to cover their eggs in feces. This trait may have evolved only once shortly after their split from their closest common ancestor and been lost in most species, or multiple times across taxa (Fig. 1). The prevalence of fecal use in so many leaf beetle subfamilies (~9/11) may indicate that this trait, or at least the propensity to evolve this trait, is ancestral in this family. Fecal retention may have evolved multiple times in association with the evolution from an ancestor that used concealed habitats to a derived state of using more open ones, such as leaf surfaces, (at least in larvae) (Vencl and Morton 1999, Chaboo 2007, JB Karren personal communication). Indeed, lineages of leaf beetles often described as basal have subterranean, aquatic, mining, or galling larvae and do not use feces in an architectural sense.

Toxins in the feces of some galeuricines protect nearby larvae, although the larvae do not carry them (Müller and Hilker 2004). Some criocerinae carry loose, wet feces on their dorsum as larvae and possess a dorsal anus to aid this behavior. Species in two genera of Alticinae also cover their eggs and/or larvae in wet fecal material (Evans et al. 2000, Jolivet and Verma 2002), but it is unknown if they share the same morphological feature. Loose, wet fecal coverings in alticines are known to protect larvae from ant attack (Di Giusto et al. 2001), but the defensive mechanism may be reliant on chemicals derived from the host plant rather than the feces itself (Morton and Vencl 1998, Vencl and Morton 1998). One chrysomeline larva builds a tubular shelter out of feces that is attached to the host plant (Jolivet and Verma 2002). The larva typically remains

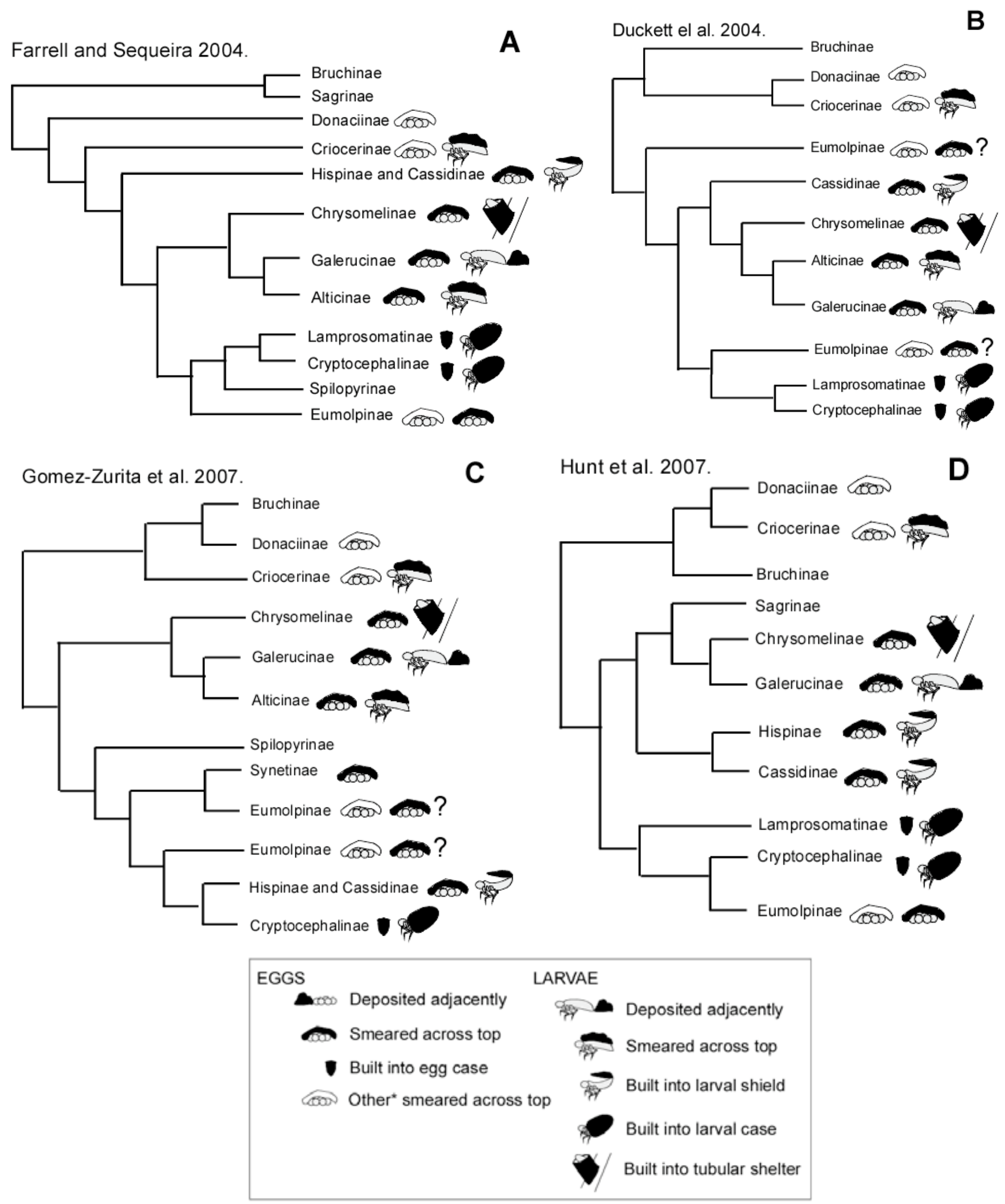

Figure 1-1. History of fecal retention in the subfamilies of Chrysomelidae. Relationships of the subfamilies are redrawn from phylogenies in the publications listed above each tree: A) From Farrell and Sequiera 2004, B) from Duckett et al. 2004, C) from Gomez-Zurita et al. 2007, D) from Hunt et al. 2007. Use of fecal material from Isono 1988, Schmitt 1988, Olmstead 1994, Vencl and Morton 1999, Jolivet and Verma 2002, and Müller and Hilker 2004. Although the relationships among the groups vary, each indicates that covering the eggs in waste material is prevalent throughout the leaf beetles and may have evolved once, possibly basally to the family. Fecal coverings in larvae, however, may have evolved four separate times: twice as a wet mass over the dorsum (Criocerinae and Alticinae), once as a fecal shield (Cassidinae), and once as a fecal case (Camptosomata: Lamprosomatinae and Crpytocephalinae). ? next to Eumolpinae egg coverings indicate that I do not know where the species that possess the trait appear on the phylogeny.

facedown inside the tube. This is a remarkable self-supporting structure and apparently unique to this group.

The most diverse fecal structures of the chrysomelids are the shields and thatches of the subfamily Cassidinae sensu lato (Fig. 1). Larvae in this group often retain both shed exuviae and feces on projections from the abdomen known as *urogomphi* or *furca*. These "shields" often take the form of unstructured masses (Chaboo 2007), but can be woven into basket-like shapes that nearly cover the entire larval body (Eisner and Eisner 2000). Feces are applied to the growing shield with a telescopic and quite maneuverable anus. The fecal shields on the tip of the abdomen are often shifted and oriented in the direction of disturbance, which is detected by tactile stimulation of lateral setae and projections (Eisner et al. 1967, McBride et al. 2000) or visually (Chaboo 2002). Shields of a variety of species have been tested for a protective function and are known to be used as a physical barrier, both by blocking attack and thrashing attackers (Eisner et al. 1967, Olmstead and Denno 1993, Müller 2002), and as a chemical deterrent (Vencl et al. 1999, Vencl et al. 2005). Cassidines can rebuild their shields if removed, and repair damaged shields. Fecal shields are probably a synapomorphy of this group and Chaboo (2007) claimed they evolved two times from a basal leaf-mining hispinoid-like ancestor (Fig. 1).

The most architecturally complex fecal structure in leaf beetles, however, would have to be the fecal cases of the group Camptosomata, consisting of the subfamilies Lamprosomatinae and Cryptocephalinae (formerly Chlamisinae, Clytrinae, and Cryptocephalinae) sensu lato. Fecal cases are bell-shaped and completely surround eggs and almost completely surround larvae (there is an opening at one end through which the head and legs are extended for walking and feeding). They take a variety of shapes and may include mineral and plant-derived components (Erber 1988, Chaboo et al. 2008). Unlike fecal shields, these structures are not maneuverable and are not rebuilt if removed (Donisthorpe 1902, Briggs 1905, Müller and Hilker 2004, Brown and Funk 2005, Chapter II), but they can be repaired. Fecal cases begin as an egg covering that ovipositing females build out of many individual fecal plates around each egg. Immediately after producing an egg, the female grasps it in her hind legs and attaches fecal plates with abdominal appendages, building walls around it and eventually creating a complete case (Fig. 1). The larva later breaks open the egg case roof, but it does not discard the egg case. Instead it turns it over and carries it on its back throughout the juvenile stages, adding to it in rows rather than plates via an anus that is positioned near the opening (Erber 1988, Brown and Funk 2005, Chapter II). Eventually the case is rounded out and the opening is sealed with more fecal material. Pupation then takes place inside the sealed pupal case. Adults emerge by cutting a circular line around the apex of the case and removing a "pupal cap" to escape.

With the exception of burrowers, animal architects often lack building-specific morphologies (Hansell 2005, 2007). Interestingly, like some other chrysomelids (in which species possess a dorsal anus or *urogomphi*), the Camptosomata possess several morphological synapomorphies associated with building and maintaining their fecal structures (Chaboo et al. 2008). (I am not sure what makes the fecal-retaining lifestyle special in this way, but it may relate to the intimacy between the builder, secretion of materials, and the building itself.) Adult females in the Camptosomata possess a complex structure of scleritized plates and muscles inside the abdomen with which the fecal plates are pressed and extruded, called the *kotpresse*, and beautifully illustrated by Erber (1968). Crytocephalines, but not lamprosomatines, also have a dip or *fovea* on the ventral side of one posterior segment of the abdomen that is surrounded by brush-like setae, into which the egg is placed while the egg case is built. Hairs on another extendible segment may serve to indicate to the female when the egg case wall has been extended far enough and construction of the egg case roof (or sealing of the egg case) can begin (Brown and Funk 2005, Chapter II). Larvae are U-shaped and bent such that the anus is near the mouth, granting larvae easy access to their building materials. Larval camptosomates also have longer legs, which must be extended below and beyond the case when walking, and fewer setae, spiracles, and less scleritization than related beetles (Moldenke 1970, LeSage 1982, Root and Messina 1983), perhaps relying more on the case for protection from predators or to regulate homeostasis than their own physiology. Although they have fewer setae, they do possess many "spiny or rounded protuberances" that could aid attachment of the larvae to their cases (Root and Messina 1983). It seems plausible that adults and larvae have a specialized internal organs that create and store the right mixture of feces, plant material, and chemicals for fecal construction. Female *Neochlamisus* have proportionately longer abdomens than males (Adams and Funk 1997), which in many insects reflects selection for increased fecundity, but in camptosomates may also be involved in fecal plate formation and storage of materials. Larvae may possess special glands that produce some kind of adhesive secretion to mix in with their feces that help attach new rows to old rows (Erber 1988). Contrastingly, fecal coverings also offer a place where larvae of some leaf beetles are known to store toxic chemicals, without developing special glands for this purpose or requiring a wounding event for their production (Hilker 1992). In fact, adult camptosomates have a reduced set of defensive glands compared to some leaf beetles (Deroe and Pasteels 1982). If this is also true of their larvae, it may be evidence that the casebearers are indeed relying on their cases for storage of chemicals and protection from predators. The chemical make-up of any of the camptosomate fecal cases is, however, unknown.

In the beginning…

Exploring the many examples of extant insects that use feces could shed light on how other insects may have evolved to use feces as building media. Egg coverings and temporary larval coverings exist in several insect taxa, including grasshoppers (Gangwere 1993), hymenoptera (Seymour 1974), lepidoptera (Bernays and Woods 2000), and many different families of beetles (Weiss 2006), indicating that this behavior has evolved multiple times. *Manduca sexta* larvae are known to excrete feces and cover their backs with waste when parasitoids are nearby, indicating a protective function (Bernays and Woods 2000). Seymour (1974) found that sawfly larvae cover their venters with wet fecal material when the temperature is raised above a certain threshold and other measures fail to lower internal temperature.

More specifically, carrying a fecal case is an ancient trait whose evolution has not been well explored. Leaf beetle larvae possessing fully developed fecal cases are known from Dominican amber (Poinar 1999, Grimaldi and Engel 2005), a rare example of behavior in fossils, and may be as old as 45 million years (Chaboo, Engel, and Chamorro-Lacayo in prep). Despite similarities to other leaf beetle fecal structures, especially the fecal thatches of *Hemisphaerota*, which completely cover the larva's body, fecal cases are probably not derived from them (Fig. 1). Instead, the wet coverings in Criocerinae and Alticinae, the fecal shields of the Cassidinae, and the fecal cases of the Camptosomata probably each evolved independently from the other, although they could have all been derived from an egg-covering ancestor. The morphological adaptations within each group do not suggest similar evolutionary trajectories. For instance, it is unlikely that a given lineage that developed a dorsal anus to aid covering itself in loose material would later evolve a curved abdomen with the anus next to the mouth to build a hardened case. By most accounts, the Camptosomata are monophyletic (Fig. 1), and it is likely that fecal cases evolved only once.

It seems that no species in the Camptosomata have lost the case-carrying trait, whereas some cassidine species no longer carry a shield (Chaboo 2007). (Although Erber [1988] states that species of *Labidostomis* and *Temnodachrys* may cover eggs only in "anal secretion" with no fecal component, this seems inaccurate [Wasowska 2007], especially since larvae of *Labidostomis humeralis* still carry a normal-looking fecal case [Erber 1988].)

More intriguingly, how did a larval case evolve out of a fecal egg covering in the Camptosomata, since other species possess larval coverings with no egg covering or egg coverings that are not incorporated into larval coverings? Often, architectural behaviors have no extant intermediates, such as in palisades moths and whorl moth caterpillars, in which only one species of the genus expresses the building behavior. Fortunately, a potential evolutionary pathway for fecal cases is apparent in other leaf beetles. One cassidine species, *Chelymorpha cassidea*, lays its eggs atop long stalks, as do many camptosomates. The larvae then feed below these stalks in groups until they are able to build their shields (Olmstead 1994). Illustrating an even more likely evolutionary pathway, larval *Lilioceris subpolita* apparently carry their eggshells for the first day after hatching. Only later do they discard the eggshell and begin covering their backs with feces (Schmitt 1988). Mothers of this species apparently do not coat the eggs with feces, but it is easy to see how similar behaviors in a group that had already evolved egg coverings could be taken up and extended by larvae to become the fecal cases of the Camptosomata.

Although not numerically diverse by leaf beetle standards, the Camptosomata (~4000 species) (Chaboo et al. 2008) can be found in ecologically diverse habitats (Erber 1988, Jolivet and Verma 2002) and there are interesting differences and similarities of fecal-related traits within and among the tribes of this group. Based on examples of cases in the literature, the fecal cases within the subfamily Cryptocephalinae are quite similar in appearance, with the main exception of a few species within the Chlamisini (although fecal addition by larvae may vary and some of the components of cases differ across tribes) (see Fig. 17 in Erber 1988). Unusually, *Coleothorpa dominicana franscicana*, a clytrine, builds a cone surrounding the plant stem on which it lays its eggs (Slosser 2003). The cone opens toward the base of the branch and may protect the eggs from passersby, much like the plastic cones that my grandfather put around his birdfeeder to keep the squirrels out of the birdseed. I am not aware of any other camptosomate building structures separate from the case. See Erber (1988) and Chaboo et al. (2008) for more comparisons of case components throughout the group.

Within the Chlamisini, however, the shape and development of fecal cases can be dramatically different. For instance, *Neochlamisus* (studied in this dissertation) and

Exema produce fecal cases similar to other camptosomates: i.e., an egg case composed of many individual plates that becomes incorporated into a relatively simple smooth tube (i.e., the larval case) with fecal additions in two planes, represented by the dorsal rim and the ventral suture (Erber 1988, Brown and Funk 2005, Chapter II). Brown (1943) and Karren (1972) consider fecal cases to be too similar among North American chlamisines to offer anything of value as characters. However, species from two related South American genera, *Fulcidax* and *Chlamisus*, construct cases much differently than what is known for *Neochlamisus*. For instance, neither genus seems to employ a ventral suture to add width to the case. *Fulcidax monstrosa*, the largest extant chlamisine, does not wrap its eggs in plates of fecal material, but rather covers them in one continuous strip, forming a spherically-shaped, rather than bell-shaped, case (Flinte and Macêdo 2004b). The larvae add to this case in large bands that extend out from the previous material, forming several flanges that spiral in different directions. The maternal portion is never dissected, as in *Neochlamisus*, and remains as a distinct ball on the back of the larval case. The finished case is quite irregular in shape, rather than a smooth tube. The life cycle of *Chlamisus minax* is nicely illustrated by Reu and Del-Claro (2005). The eggs of this species are wrapped in plates, but the egg case still takes the form of a round ball. This maternal construction is again not altered, but larvae add rows of increasing size in one direction from it, forming a long cone. Prior to pupation, a much wider section is added to the bottom of this larval cone, and the previous sections are cut off and discarded. I have never seen another example of parts of a larval case deliberately removed by larvae, although Donisthorpe (1902) says that the maternal portion of the larval case of one clytrine breaks off and the larva seals the remaining hole.

The adults of the Chlamisini share the same “warty” appearance unique to the tribe, and many authors consider the tribe monophyletic (Karren 1972, LeSage 1984, Reid 1990), perhaps indicating that changes in building behaviors may be freer to diversify than adult morphology. There is no phylogeny of the genera within this tribe, but it might be assumed that the style of case building in *Fulcidax* and *Chlamisus* is extremely derived from the basic pattern seen elsewhere. Considering only the structurally "simpler" method of egg wrapping seen in *Fulcidax*, this trait may seem basal, but considering the shared traits of *Neochlamisus* and *Exema* cases with the rest of the camptosomates, this is phylogenetically implausible. This means that *Fulcidax* evolutionarily lost the fecal plates made by ovipositing females. The larval cases of some species of the phylogenetically ambiguous camptosomate subfamily Lamprosomatinae appear tube-like, as in most Cryptocephalinae (Kasap and Crowson 1976), while others appear more like *Fulcidax* cases with larval additions added to two lateral flanges (Erber 1988).

The chlamisines, and probably the majority of the camptosomates, are mostly found in the neotropics and may have originated in South America, although they are now found in all zoogeographical areas (Karren 1970). Most camptosomate adults are herbivorous, as are most chrysomelids, but the majority of larval camptosomates are saprophagous or fungivorous, which is quite unusual (Riley 1874, Donisthorpe 1902, LeSage 1984b, Erber 1988, Jolivet and Verma 2002). Many of the Cryptocephalini live in the leaf litter and apparently cannot climb plants (LeSage 1984b, 1985, 1986), whereas most chlamisines are intimately tied to and dependent on their host species (Brown 1943, LeSage 1984a, Funk 1998, Flinte et al. 2003). Many species of clytrines are myrmecophiles, meaning they live inside ant nests as larvae, and some are even willing and able to eat dead arthropods, possibly including their insect hosts (LeSage and Steifel 1996). Some authors have proposed that these egg cases are attractive to ants, which induced to bring them into the nest, either through chemical manipulation or by mimicking normal foods or material for nest construction (Donisthorpe 1902, Erber 1988). Results from choice tests are mixed (LeSage and Stiefel 1996, Stiefel and Margolies 1998), but fecal cases would most assuredly help the beetle survive the trip, thus allowing it access to a new niche. Adults apparently drop eggs near ant nest openings and some adult clytrines may even mimic the ants themselves (Selman 1962), perhaps to avoid being eaten by their hosts as they leave the nest (Donisthorpe 1902, Erber 1988). The ground-dwelling taxa have straight tarsal claws and often retreat inside their cases when disturbed. The case falls to one side, but the larva's flattened head blocks the entrance (Donisthorpe 1902, Lawson 1976, Erber 1988). In Chlamisini however, falling from the host plant could be fatal, so larvae have recurved claws that dig into leaf tissue and they hold their cases down to the surface when provoked, covering the entire body, legs included. (Karren 1964, Wallace 1970, LeSage 1984a, Neal 1989, Brown and Funk 2005, Chapter II).

Kasap and Crowson (1976) created a phylogenetic dendrogram of the Camptosomata using 19 morphological and ecological characters, although LeSage (1985) found fault in one of their trait delineations. They conclude that the subfamilies Lamprosomatinae and Sphaerocharinae (now included within Lamprosomatinae) are basal to the group, with Chlamisini branching off next, and Cryptocephalini and Clytrini splitting last (Fig. 2). This tree might indicate that wrapping eggs in individual plates and

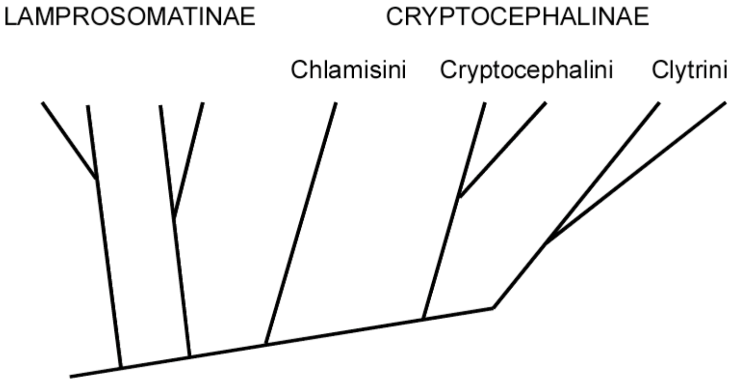

Figure 1-2. Relationships among camptosomate groups. Cladogram redrawn from phylogenetic dendrogram based on 18 morphological characters and 1 ecological character (association with ants) of larvae and adults of the Camptosomate group in Kasap and Crowson 1976.

the tube-like larval case structure are derived; unfortunately no case-associated morphological or behavioral traits were included in their diagram. If true, it would also suggest that living in ant nests is derived, perhaps meaning that this lifestyle is facilitated by the fecal case, rather than vice versa. Of course a phylogeny of the Camptosomata using molecular data from many species within each tribe, especially those whose cases are described in detail, would be most helpful.

Knowledge of the evolution of other building components also begs for more information. Firstly, new material not only needs to stick to itself as it is added, but each row of newly added fecal material must stick to the previously applied one. This is true for adult egg case building and larval case building. The attachment point between the rows that larvae add is a particularly weak one and a source of anisotropic failure (personal observations, Hansell 2005), but the horizontally and vertically overlapping addition of plates by females to the egg case does not appear to suffer the same affect. What building traits have evolved to accommodate these factors? Secondly, larvae of the casebearer *Neochlamisus* emit some kind of liquid from their mouths (Brown and Funk 2005, Chapter II) (perhaps a hypopharnyx secretion) and/or anus (Briggs 1905) when disturbed. This substance then hardens into a dark caramel-colored solid, similar to the color of finished cases. If camptosomate larvae incorporate a fixing agent into their feces before applying new layers (as suggested by Riley 1874, Donisthorpe 1902, Briggs 1905, Erber 1988, Brown and Funk 2005), this substance may be derived from a similar secretion. It would be interesting to know the make-up of this substance and to determine if it is incorporated into new layers of the case (representing a predaptation if it originated for defensive purposes), if it is incorporated into the maternally formed plates (indicating an anatomically different origin since adults do not use their mouthparts to build egg cases), and if it is host-plant derived. Erber (1988) says both larvae and adults possess rectal glands with which they create an adhesive secretion used in case-building, indicating potentially similar anatomy for this "glue", but a different one for the defensive spitting.

The egg stalk found in some casebearers (including species of Clytrini and Lamprosomatini, *Exema* and *Neochlamisus*) is also intriguing. Similar strands holding up stenogastrine wasp nests, lacewing eggs, and many spider egg sacs are considered as defensive, preventing attack from ants (Hansell 2005), but *Neochlamisus* stalks become twisted as the mother adds rows of plates, sometimes lowering the egg case close to the leaf surface, perhaps exposing them to ants. Perhaps the egg stalk simply serves to keep larvae tied to their host plants, since leaving them would be fatal. It would be interesting to see if the presence of egg stalks in the Camptosomata is correlated with the evolution of herbivory or host plant specificity, assuming it has evolved multiple times. Alternatively, it could be basal to the casebearers and lost in nonherbivorous groups. Although not necessarily affecting fitness, egg stalks may explain different egg case structures among camptosomates. For example, perhaps the egg stalk provides stability, allowing *Neochlamisus* to build a flat egg case roof, while other species close the egg case in a more gradual way (see Lawson 1976, Erber 1988).

There is also no fungal growth on *Neochlamisus* fecal cases found in the wild, whereas those in the lab can become covered in tiny white mycelia (personal observation). Although antifungal substances may be inherent in chrysomelids or derived from the host plant, camptosomates may have evolved the production of special chemicals or even symbiotic associations for this purpose. They may have also needed to develop different mechanisms to incorporate previously evolved antimicrobial chemicals into their fecal material, such as glands in the digestive tract.

Follow the directions…

Two recent books on animal architecture, *Animal Architecture: Building and the Evolution of Intelligence* (Gould and Gould 2007) and *Built by Animals* (Hansell 2007), speculate on the cognitive capacity required to build some of the intricate structures found in nature. Although not a focus of my studies, a comparison with Camptosomates might prove fruitful. Gould and Gould state, "when it comes to building, solitary insects provide textbook examples of innate control." (p. 74). In other words, most examples from insects show that building behaviors are hardwired. This is true even in insects that are known to possess learning and processing abilities during other activities. For example, hunting wasps follow strict rules when constructing burrows and nests (Gould and Gould 2007), but finding their nests and provisioning their young requires memory (Tinbergen 1951). Hansell (2005, 2007) says that building behaviors are selected to be few in number, stereotyped and repetitive. Indeed, weaver ants build elaborate nests in a very variable environment (i.e., the tree canopy), but may only express 29 individual behaviors to do so (Wilson 1980). Caddisfly larvae can build intricate and precisely round cylinders by using one simple rule: Add grains of sand of size similar to previous grains to the least extended section of the case, using body size (or head size) as a template for case diameter (Hansell 2005, 2007). Most of the building processes in Camptosomata are probably heavily regimented in a similar way. In fact, Briggs (1905) replaced the fecal case of a fourth instar *Neochlamisus* larva with a hand-made case of paraffin wax. The larva continued to add to its case, including cutting and adding more material to the ventral suture. This indicates that any encapsulating case-like structure induces accurate building behaviors. They are probably cued to add material to any edge of material and are obviously not concerned with the content of the current case. Further, when the case is removed, larvae will sometimes begin coating the substrate in a flat layer of fecal material (personal observation). Obviously larvae are not concerned about the shape or location of this pre-built fecal edge. This may explain why larvae are incapable of building a case de novo. The rule "add material to material" requires a starting point.

For many builders, the starting point is the most difficult since there is no template and behaviors must be more flexible given variations in habitat (e.g., spider webs across bushes and weaver bird nests in trees) (Hansell 2005). For camptosomates, this may not be a problem. The egg serves as a starting point and template for females and the egg case is the starting point for larvae. Larvae then probably use their own bodies as a template for case size and roundness. Once, I disturbed an ovipositing female after she had begun building an egg case. The base and about half of the walls were completed. She walked away from her egg, turned around, and came back to it. When her head reached the egg, she ate the entire egg, leaving just the egg case. (Many insects will devour their own eggs if conditions are not appropriate for their development and the resources are better spent elsewhere. And an unwrapped *Neochlamisus* egg will not survive.) She then walked over the half-finished, empty egg case, grabbed it in her hind legs in the typical egg-wrapping position, and promptly closed off the egg case by building a roof. She walked away from what looked like a normal, completely closed egg case, only it was half as tall as it should be and it was empty. This observation indicates that females are stimulated by the presence of the egg case to finish wrapping an egg, but they use the egg itself to determine when to finish the egg case. The egg is the template for the egg case. Since the case was empty, she could no longer feel the top of the egg, and so moved to the next step in the sequence: begin closing the case.

Building a fecal case probably requires different cues for different purposes. Transitions between only adding length (as done in the beginning instars) to predominantly adding width (in the last instar) (Chapter II) may be directly cued by genetic signals and/or to larval size, which is also dependent on feeding. Chapman (1998) states that some insects perform ecdysis based on gut size. In *Neochlamisus*, the cue for ecdysis would also need to cue attaching the case to the leaf, which takes place prior to ecdysis. Neal (1989) found that when females are held upside-down, they produce egg cases that are significantly wider and shorter than in the field. This result could be a difference between field and laboratory conditions since the proper controls were not done, or could be due to differences in gravitational cues. Although spiders seem to rely on gravity when building their webs, it is not apparently necessary for proper web building (Witt et al. 1977). In *Neochlamisus'* case, orientation may more likely affect a female's ability to hold onto the egg while wrapping it properly and this gravitational requirement might explain some of the interspecific variation in case size since some species lay more eggs on the top of the leaf, whereas others lay more eggs on the bottoms (e.g., *N. platani*, which lays most of its eggs on the abaxial side of leaves, produces a more elongate egg case than related species) (Brown and Funk 2005, Chaboo et al. 2008).

Therefore camptosomate case construction might be quite innate, yet variation may reflect environmental factors.

Larval case construction actually seems to involve two distinct developmental steps. During the first few instars, the case is mostly lengthened, but during the last instar it is greatly widened into a barrel-shape in preparation for pupation (Brown and Funk 2005). Although it may maintain a general, low-angle logarithmically spiraling shape, which in other species (e.g., snails and helicopsychid caddisflies) allows volume to increase with minimal use of material and without excessive surface area, pupal cases seem to be the product of two separate logarithmic trajectories (as found in some brachiopods, Aldridge 1999) (A. Aldridge personal communication).

There are several impressive traits in *Neochlamisus* building that highlight the sensitivity and acclimatization needed to build such a delicate structure. Female *Neochlamisus* use their tarsal claws to hold onto the egg case, but never touch the more sensitive egg, which might be damaged by them (Brown and Funk 2005, Chapter II). After watching several oviposition events, I have never seen a female accidentally dislodge one of the fecal plates. This maneuverability requires quick coordination and is based only on touch, since the female is blind to the entire process, as it is facing away from the egg. Fecal cases also require two distinct behaviors that must be regularly performed: 1) attaching horizontal additions to the dorsal rim and 2) opening the ventral suture along a previously determined suture and filling in the opening with vertical additions (Brown and Funk 2005, Chapter II). The second aspect adds a new dimension not found in the simpler uniform tubes of caddisflies. Once completed, the case wall is uniformly thick across its circumference, except for a longitudinal indention at the ventral suture. This represents a thinner point in the case wall, which may make cutting through the case for further expansion easier. The fact that the case wall is otherwise of uniform thickness is amazing considering the builder does it all from an internal position. Perhaps larvae also add another coat of feces to the entire internal wall of the case, explaining its smooth internal surface (Chaboo et al. 2008). Since the case is constantly being expanded, it is unknown how often such a procedure would be necessary or what triggers it.

Neochlamisus larvae are capable of repairing damage to their cases (Briggs, 1905, Erber 1988, Brown and Funk 2005, Chapter II, see also Eisner and Eisner 2000 for repair in a cassidine), whereas repairing a structure is not guaranteed in other animal builders. Gould and Gould (2007) relate an example of a wasp that builds a large funnel out of smoothened mud to protect its egg chamber from parasitoids. Once one section of the funnel is created, the wasp will never return to it, even if it is partially damaged, or if the damage negates the function of the funnel. So it seems that like other architects, casebearers may be relying on a simple set of rules to produce their elaborate structures, but unlike some architects, they may possess multiple blueprints and are willing to alter their plans when necessary.

What's next…

My thesis focuses on the unique architectures of the Camptosomata by studying species in the genus *Neochlamisus*. My studies explore the role that fecal cases play in the interactions of larvae and pupae with their biotic and abiotic environments. Chapter II provides a detailed account of the formation of the case and its role in the life history of several *Neochlamisus* species. It provides descriptions of the behaviors of females as they wrap eggs, how larvae expand their cases, and how adults emerge from the pupal case. It also describes in detail the temporal pattern of larval and pupal development. The remaining chapters investigate potential adaptive functions of the case. My work has not evaluated the evolution of cases, beyond the speculation from literature provided here. I only test potential reasons for their evolution or certain adaptive functions they may currently provide. Chapter III examines the survival of larvae in the field with and without cases and the effects of predation and parasitism. It shows how important the case is for survival. Chapter IV more directly tests the effects of the case and its various components on preventing predation in two species of *Neochlamisus* against three arthropod predators. After discovering the importance of external trichomes embedded in the case material in lowering the threat of predation, Chapter V examines the surprising larval preference for these plant hairs, which evolved in plants in part to prevent herbivory and are usually avoided by herbivores. Chapter VI provides explicit tests for the hypothesis that fecal cases moderate the effects of various humidity conditions. Although cases are not suitable for this function, humidity was found to interact with case material in a very interesting way, which may have great implications for fitness. Chapter VII tests how fecal cases affect temperature and whether or not they may play a role in *Neochlamisus* thermoregulation. Chapter VIII describes an unusual biotic threat to *N. platani* eggs and larvae involving another animal architect. I close with a summary of these findings and their interactions as well as some other information discovered subsequently.

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CHAPTER II

ASPECTS OF THE NATURAL HISTORY OF *NEOCHLAMISUS* (COLEOPTERA: CHRYSOMELIDAE): FECAL CASE-ASSOCIATED LIFE HISTORY AND BEHAVIOR, WITH A METHOD FOR STUDYING INSECT CONSTRUCTIONS

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Abstract

Leaf beetle taxa belonging to the Camptosomata group use their fecal material to construct cases within which their larvae develop. Despite this intriguing behavior, the fine-scale natural history of very few camptosomates has been determined. Here, we present a highly detailed account of fecal case construction and associated behaviors and life history patterns in *Neochlamisus* case-bearers. This study documents diverse elements of oviposition and egg case construction, larval development and case enlargement, pupal case formation, and adult maturation and emergence. Using an independently derived method, we further document temporal and spatial aspects of larval case architecture. Assays of 10 *Neochlamisus* taxa in the laboratory are supplemented with field observations in perhaps the single most comprehensive description of case-associated camptosomate biology.

Introduction

Nearly all animals face selection pressures from predators, and innumerable antipredation mechanisms have evolved to combat such threats. Phytophagous insects, because of their slow metabolism and exposed microhabitats, may be particularly vulnerable to predation (Southwood 1973). In response, chrysomelid leaf beetles, for example, have evolved a diversity of predator-deterring tactics. These include mimicry (Balsbaugh 1988), powerful jumping abilities (Lindroth 1971), tarsal adhesion (Eisner and Aneshansley 2000), and the sequestration and secretion of toxic compounds (Pasteels et al. 1982, Ferguson and Metcalf 1985, Houghgoldstein et al. 1993), among many others. Intriguingly, various chrysomelid taxa actually use their waste material for defensive purposes. Perhaps most famous in this regard are the tortoise beetle larvae (Cassidinae) that attach fecal matter, and sometimes exuviae and plant material, to specialized structures on the abdomen, forming a shield or thatch that is held over the dorsum during larval life (Eisner et al. 1967, Olmstead 1994, Chaboo 2005). There, it serves as camouflage (Eisner and Eisner 2000) and as a physical (Hilker 1992, Olmstead and Denno 1993, Nogueira-de-Sa and Trigo 2002) and chemical (Morton and Vencl 1998, Vencl et al. 1999) barrier to predators. Compared to the tortoise beetles, however, much less research has focused on the fecal structures of the lineage in which fecal manipulation plays perhaps its largest role in chrysomelid biology.

We refer to the camptosomate chrysomelids, a group comprised of the chlamisines, clytrines, cryptocephalines, and lamprosomatines, all of which construct a ‘case’ from their fecal material, within which individual larvae live and complete their development, enlarging it as they grow. These larval cases actually begin as fecal egg cases that ovipositing females construct around each individual egg, and serve their final function as pupal cases within which metamorphosis occurs (Fig. 1). Although considerable taxonomic variation exists in their particular shape and structure (Erber 1988), in all instances these cases completely enclose the body of the larva except for an opening from which the legs and head can emerge, allowing movement and feeding. These robust cases undoubtedly provide a degree of physical protection from predators (Wallace 1970, Root and Messina 1983) and likely serve a cryptic function as well. These cases also appear to protect larvae from desiccation, to which they are more susceptible than typical chrysomelid larvae (Root and Messina 1983). However, none of these issues have been explored in depth.

Furthermore, although the chrysomelid case-bearers have long attracted the interest of naturalists (Riley 1874, 1882, Marlatt 1888, Popenoe and Marlatt 1889, Cockerell 1891), the systematic and detailed study of case-associated biology is minimal for most groups. Some workers have described larval and case morphology in particular taxa (e.g., see LeSage's 1984b, 1985, and 1986 studies of crytocephalines). And Erber (1988) established commonalities among the life histories and details of case construction of one clytrine, one cryptocephaline, and one lamprosomatine species. With respect to the remaining group of case-bearers, the chlamisines, previous workers have also evaluated particular aspects of biology for certain taxa (Brown 1943, 1946, 1952, Karren 1964, Wallace 1970, Karren 1972, LeSage 1984a, Neal 1989), with some of the most complete behavioral observations having been made a century ago (e.g. Briggs 1905).

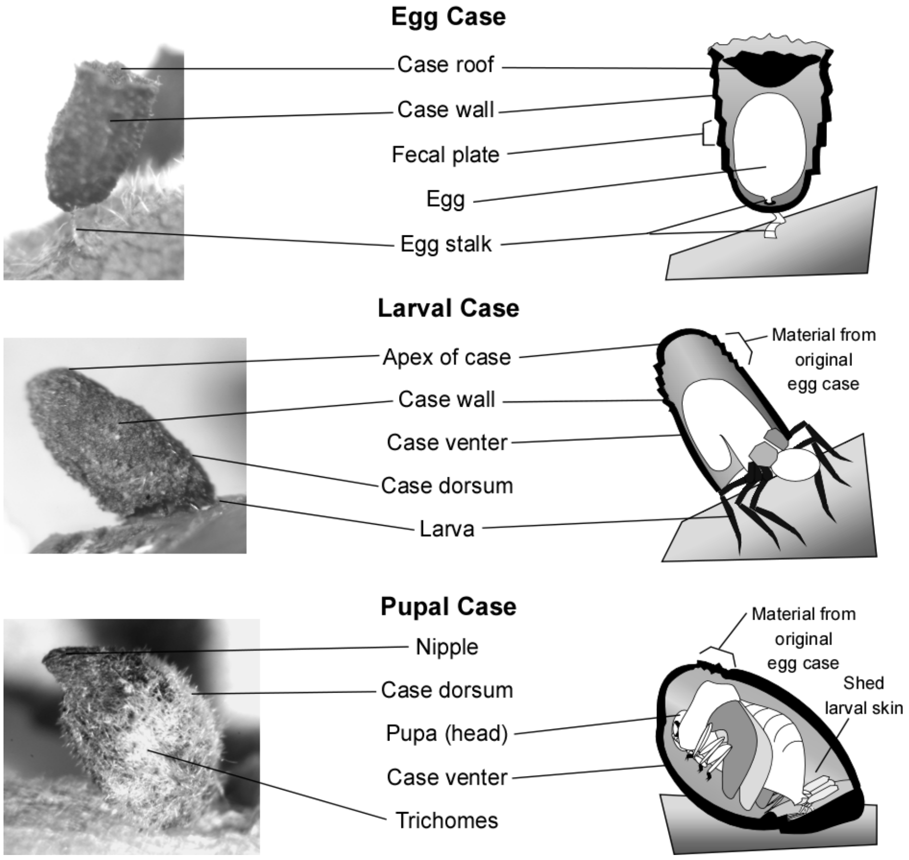

Figure 2-1. Photographs and diagrammatic illustrations depicting external and internal aspects of *Neochlamisus* cases across life history stages.

In this paper, we present perhaps the single most comprehensive study on the case-associated life history and behaviors of any case-bearing leaf beetle taxon. We also present an independently derived method for the study of case construction and architecture during larval development (see also Briggs 1905). The subjects of our studies are taxa from the eastern North American *gibbosus* species group of the chlamisine genus *Neochlamisus* Karren (Table 1). (Until *Neochlamisus* was defined by Karren (1972), the *gibbosus* group species were variously assigned to *Arthrochlamys*, *Chlamisus*, and *Chlamys*.) These ca. 3 - 4 mm beetles have univoltine life cycles that include an adult winter diapause. Unlike many other camptosomate taxa, whose larvae feed on detritus and leaf-litter (Riley 1882, LeSage 1985, 1986, Stiefel 1993), previously studied chlamisine taxa are true herbivores that feed and complete development on their host plants. In *Neochlamisus*, these host associations are often quite specialized and most species primarily use but a single host plant genus or even a single species (Brown 1943, 1946, 1952, Karren 1972). An exception to this rule is provided by *N. bebbianae*, which is known to feed on particular species from six tree genera representing five different plant families (Brown 1943, Karren 1972, Funk 1998). *Neochlamisus bebbianae* populations associated with each of these plants are hereafter referred to as separate “host forms” and are the subject of ongoing studies with respect to host-associated speciation (Funk 1998, Funk et al. 2002). The present study involves laboratory assays of four *N. bebbianae* host forms and six other *Neochlamisus* species. These are supplemented by field observations based on these and other *gibbosus* group taxa (Table 1).

Table 2-1. Host plants and relative frequency of plant part usage by highly studied *Neochlamisus* taxa.

Taxa	Primary host	Oviposition sites	Larvae found on:	Pupation sites
N. bebbianae				
alder host form	*Alnus* spp. = alders (Betulaceae)	center of upper leaf > stems	lower leaf > apical meristem > stems	stem > apical meristem, lower leaf > dried leaves
birch host form	*Betula nigra* = river birch (Betulaceae)	center of upper leaf >> stems	apical meristem > lower leaf	lower leaf ≥ apical meristem
maple host form	*Acer rubrum* = red maple (Aceraceae)	lower leaf, generally on major leaf vein > petioles	lower leaf >> stems	Stem ≥ lower leaf
willow host form	*Salix bebbiana* = Bebbs willow (Salicaceae)	center of upper leaf >> lower leaf	apical meristem > lower leaf	apical meristem, stem > lower leaf
N. bimaculatus, N. eubati, N. gibbosus	*Rubus* spp. = e.g.., blackberry (Rosaceae)	upper and lower leaf > stems >> flowers	apical meristem > lower leaf >> upper leaf	lower leaf, apical meristem
N. chamaedaphnes	*Chamaedaphne calyculata* = leather leaf (Ericaceae)	center of upper leaf	apical meristem > lower leaf	apical meristem > lower leaf
N. comptoniae	*Myrica asplenifolia* = sweet fern (Myricaceae)	stems > lower, upper leaf	lower leaf	lower leaf
N. platani	*Platanus americanus* = sycamore (Platanaceae)	lower leaf > upper leaf, stems >> fruits	lower pubescent leaves – often at base of major leaf veins	lower leaf >> stem

Note: Relative usage patterns derive from field observations by DJF. “Upper leaf”, “lower leaf” = upper, lower leaf surfaces, respectively.

Materials and Methods

Study animals

All study animals were collected on their host plants in the field in June/July of 2004, or derived from eggs laid by field-collected females. Collection localities are as follows: *N. bebbianae*: alder host form: (White County, GA), (Orleans County, VT); maple host form: (Greene County, GA), (Rockingham County, NH), (Suffolk County, NY); birch host form: (White County, GA); willow host form: (Cumberland County, ME), (Caledonia County, VT). *N. bimaculatus*: (White County, GA), (Davidson County, TN). *N. chamaedaphnes*: (Suffolk County, NY). *N. comptoniae:* (Rockingham County, NH). *N. cribripennis*: (Suffolk County NY). *N. eubati*: (Rockingham County, NH). *N. platani*: (White County, GA), (Davidson, Williamson Counties, TN).

All assays were conducted between June and September of 2004 at Vanderbilt University, in Nashville, Tennessee. Larvae and adults were generally maintained in groups of 1-5 individuals in 5cm plastic Petri dishes that were lined with moistened filter paper and provided with foliage of the native host plant. Dishes were cleaned and fresh foliage provided at regular intervals. Dishes were stacked in clear plastic boxes that were lined with moistened paper towels, sealed with plastic wrap and the box lid, and maintained in lab incubators at ca. 27C, 64 ± 35 % humidity and a 14:10 light:dark schedule. There were a few deviations from this protocol: Eggs and pupae were maintained in groups of 50-100 and 1-10 individuals, respectively, in dishes with dry filter papers to avoid fungal growth during these sessile stages, and adults were sometimes maintained *en masse* within boxes or in mesh bags secured to host trees in a greenhouse. Additionally, five larvae were reared under a 24:0 light:dark schedule in one assay of case construction.

Oviposition and egg case construction

Several instances of *N. platani* oviposition and egg case construction were observed under a dissecting microscope. These activities were documented with the help of a microscope-mounted CCD video camera and an SLR digital camera. Case architecture was evaluated by dissecting completed egg cases.

Larval biology and a method for studying case construction

Observations on *N. bimaculatus* larvae illustrated the processes by which larvae free themselves and begin to use their egg case as a new home. Additionally, one individual each of *N. bimaculatus* and *N. platani* was daily observed and weighed while in its case in order to provide detailed information on growth rates and the number and timing of molts. These latter assays began the day each animal emerged from its egg case and concluded when the case was sealed for pupation.

Neochlamisus larvae enlarge their cases as they grow by the addition of new fecal material. A major emphasis of this study was on the behavioral, spatial, and temporal elements of this larval case construction. To evaluate these processes, we developed a technique for this study that may prove to be of more general use in investigations of insect-constructed architecture. We began by applying Testors paint (Rockford, IL) to the entire case on the day a larva emerged, before it had added any new case material. The method thereafter involved two daily steps. First, the amount of fecal material added to a larval case over the preceding day was measured under a dissecting microscope fitted with an ocular micrometer (Fig. 2A and B). Second, paint was applied only to the newly added portions of the case, using a sharpened toothpick (Fig. 2C and D). Across days, these regions were painted in a specific color sequence (red → blue → white → green → orange → purple → yellow → red….).

The temporal pattern of larval case-building activity and the changes in case size and shape across larval stadia were documented by plotting the amount of fecal addition to two different regions of the case (discussed in Results) as a function of time. By daily examination of the distribution of colors over the case, the precise spatial pattern of additions and the fate of early-added material could also be documented. These studies examined hatchlings of eight willow host form larvae plus one *N. comptoniae* larva, which was painted but not measured. Additionally, 16 *N. eubati* larvae were studied, but painting of these was not initiated until the third or fourth larval instar. Five of the *N. eubati* larvae were maintained under conditions of continual light to explore the possible existence of intrinsic rhythms in case construction behaviors. Painting did not appear to hinder larval activity or case construction as long as the paint was kept away from the larva itself. However, painted individuals did develop somewhat more slowly than beetles reared in the past, possibly due to their prolonged removal from food and optimal conditions during daily manipulations.

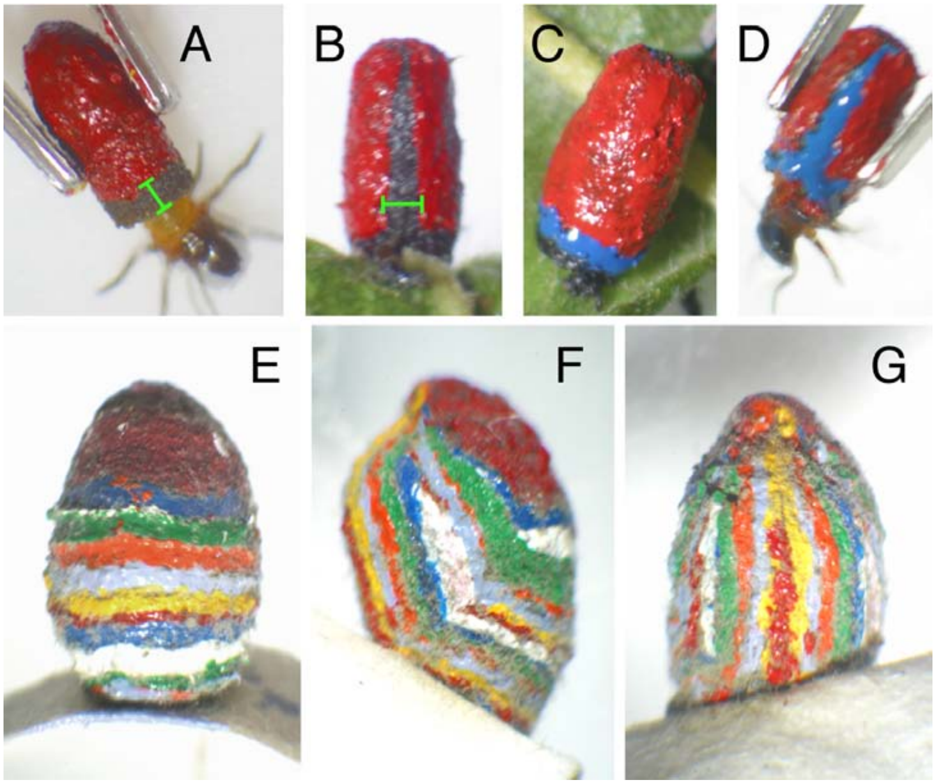

Figure 2-2. Method for studying case construction. Photographs illustrate larval additions to, and prior painting of, (A-D) a young *N. eubati* larval case and (E-G) an *N. bebbianae* willow host form pupal case. (A) Addition to dorsal rim of case. (B) Addition to region of ventral suture. In both photos, dark parts of case are fecal material added in the 24 h after the case was completely painted red. Brackets indicate where measurements were taken for this study. (C and D) The next step: painting newly added material a new color. (E) Dorsal, (F) lateral, and (G) ventral views of pupal case, illustrating many days of case additions and subsequent painting.

Pupation and adult emergence

Last, we evaluated the temporal pattern of activities following the larva's formation of its 'pupal case', namely, pupation, adult eclosion and subsequent maturation, and actual adult emergence from the case. This was accomplished by using a razor blade to carefully cut and remove case material leaving a small (approximately 3 x 4 mm) 'window' in the side of each pupal case, and thereafter making daily observations on the development of the animal inside. Study animals included eight of the *N. eubati* from the case-painting studies. For these animals, windows were added 9-16 d after the formation of the pupal case. Additionally, five willow host form individuals were observed, with windows added 1-2 d after pupal case formation. Most of these pupal cases contained either a pupa or a teneral adult, which do not participate in case-construction. However, in some instances windows were cut into cases with larvae that had not yet pupated, and these are capable of repairing case damage. Therefore, some windows had to be reopened or widened one or two times preceding the pupation of these larvae.

Additional observations

A variety of other pertinent biological observations are raised in later sections of this paper. Some of these were initially made during the 2004 assays and others corroborate previous unpublished findings. Additionally, DJF has made diverse observations on *Neochlamisus* biology over ten years of studying these animals in the laboratory and the field, many of which are published here for the first time. Otherwise unattributed observations on *Neochlamisus* fall into these categories.

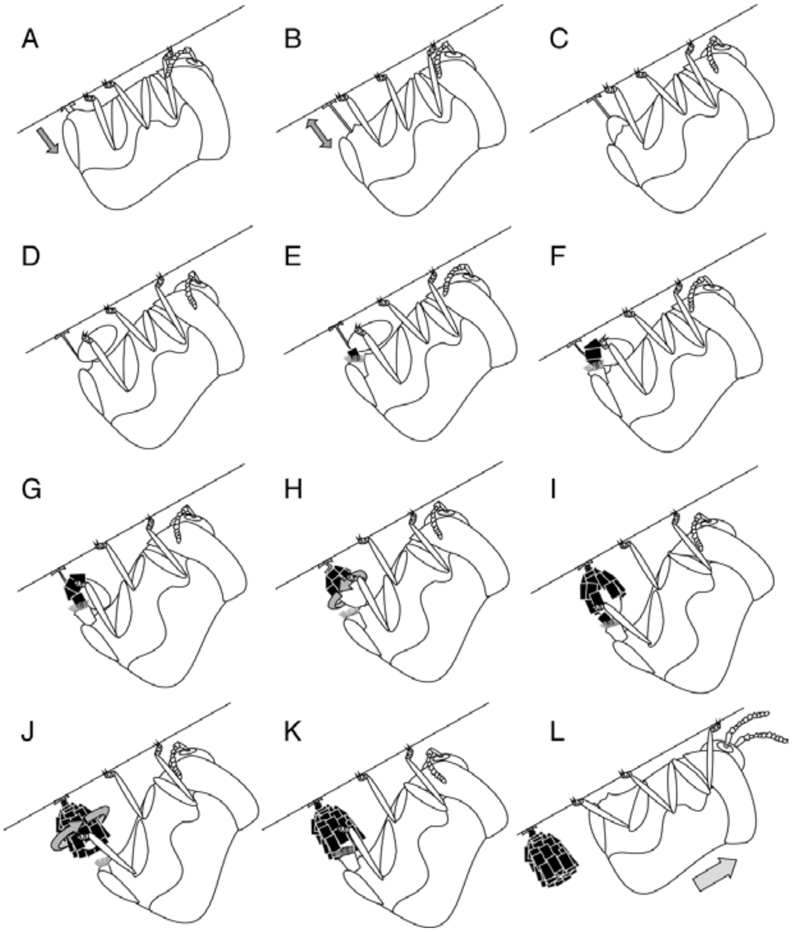

Figure 2-3. Oviposition and egg case construction. (A) Deposition of pliable substance onto leaf surface. (B) Stretching of substance into stalk via abdominal movements. (C) Egg emergence. (D). Egg held parallel to female's body. (E) Placement of first fecal plate. (F) First row of plates is completed. (G) Attachment of plate to previous row. (H) Turning egg counterclockwise or (J) clockwise after each plate attachment (G,I). (K) Egg case roof formation. (L) Female abandonment after case completion.

Results and Discussion

Oviposition and egg case construction

Lab observations. Based on our observations of *N. platani* females, the process of oviposition and egg case construction proceeds as follows (Figs. 3 and 4A-G): After identifying an acceptable oviposition site, the female feeds superficially on the plant surface, removing only the upper layer(s). She then turns 180 ° and begins to repeatedly extend and retract her posterior abdominal segments over the place of feeding while depositing a clear, pliable substance that conforms to the leaf surface (Fig. 3A). This substance is pulled by abdominal movements into a stalk-like structure, which is connected to the single, orange, ovoid egg that subsequently emerges from the female (Fig. 3B and C). We suspect that the material of the egg stalk may additionally form a coating that surrounds the entire egg.

This new egg is immediately held by the tarsal pads of both metathoracic legs, with the tarsal claws lifted away from the egg surface (Fig. 3D). Holding the egg parallel to her body, the female then initiates case construction at the egg base, where it attaches to the stalk. One by one, she extends from her abdomen thin square plates that are largely formed internally by rectal sclerites and muscles. These plates are composed of fecal material combined with secretions from special anal glands (Karren 1972, Erber 1988). Each plate is extended and attached with the abdomen while being smoothed and compressed by the genital segments and associated rows of golden bristles held like thin brushes on either side of the plate (Figs. 3E and 4C). Between plate additions, the female moves these bristles along the surface of the egg case, perhaps using them as sensory structures and to help compress plates. Formation and attachment of each plate takes 20-30 s.

During the addition of plates, the tarsal claws are used to hold the egg case, but never touch the egg itself. Following the addition of each plate, the female uses her metathoracic legs to turn the egg perpendicularly to its long axis in either a clockwise or counterclockwise direction (Figs. 3H, J, and 4D). This direction is typically reversed after the addition of approximately three plates and three turning bouts, perhaps due to tension on the egg stalk. The number of degrees the egg is turned is variable. Due to the repeated turning, the egg stalk becomes twisted and shortened, causing the egg case to rest closer to the leaf surface (Fig. 3I).

During these activities, the egg is stabilized by resting in a dimple-like fovea that is bordered by two short rows of bristles on the female's fifth abdominal sternite. In this position, individual plates continue to be added, until the egg base becomes encircled in a cup-like arrangement of contiguous plates, the only plates that actually (basally) touch the egg (Fig. 3F). After the first row is finished, the angle between the egg and the female is gradually increased to 90 degrees, at which point the apex of the egg rests in the female's fovea (Figs. 3G, 4A, and B). Individual plates continue to be added, one row at a time, creeping up the long axis of the egg (Fig 3I). Within a row, plates are seemingly placed haphazardly around the circumference of the case, with spaces initially between them, rather than being added in orderly fashion with each placed immediately next to the preceding plate. Plates in a new row are vertically joined to the distal half of plates from the previously completed row such that later rows eventually rise above and away from the egg (Fig. 3J). Once the growing edge of the egg case rises one or two plate lengths

Figure 2-4. Aspects of the case-associated biology of *Neochlamisus*. (A-E) *N. platani* constructing egg case. (C) Abdominal segments showing bristles. (D) Turning egg case. (E) Adding to roof. (F) Newly completed *N. platani* egg case, still moist and green. (G) *N. bimaculatus* dried egg case. (H) *N. bimaculatus* larva emerging from egg case. (I) Alder host form larva with case removed. (J) Willow host form first instar larva. (K) *N. cribripennis* fourth instar larva and associated feeding damage on *Vaccinium* leaf. (L) Cutaway view of *N. bimaculatus* larva in case sealed to the substrate during ecdysis, assuming inverted position. (M) Ring of fecal material left behind by willow host form larva after cutting free its case from the substrate following molting. (N) Sclerotized portions of shed exuvium left within ‘fecal ring’ after molting by alder host form larva. (O) Willow host form larva repairing damaged case by adding fecal material. (P) Willow host form larva exhibiting trichomes added around case opening during previous 24 h. (Q) *N. chamaedaphnes* pupal case on non-host plant (a sedge), illustrating wandering by mature larvae; also note bands of alternating colors in case, reflecting addition of separate fecal layers. (R) Maple host form pupal case on host plant, illustrating possible crypsis. (S) Cutaway view of maple host form pupa in case. (T) *N. bimaculatus* teneral adult in pupal case. (U) *N. bimaculatus* adult prior to emergence from pupal case, with case cut away to illustrate tight fit. (V) Maple host form adult cutting pupal cap from within case. (W) Abandoned, trichome-encrusted maple host form pupal case and cap. (X) *N. comptoniae* adult emerging from pupal case. (Y) Cross section of trichome attic removed from apex of *N. platani* case. (Z) Adult *N. bimaculatus.*

beyond the top of the egg itself and the egg is no longer in contact with the female, she begins to form the roof of the egg case (Fig. 3K). To do so, she adds another row of plates in the manner described above, but this time uses her abdomen to bend each of them to a 90-degree angle so their distal halves point toward the center of the case. After a ring of these bent plates is formed around the circumferences of the case wall, additional plates are added that seal (Fig. 4E) and greatly thicken (Fig. 1A) this case roof, resulting in a flat-topped case (Fig. 4G).

No dropping or dislodging of plates by females was observed during any case-building process, even though the plates appear to be loosely attached and the female manipulates them blindly. This may attest in part to the great adhesiveness of the plates. Each case requires approximately 75 to 100 plates and 25 to 50 m to complete at 20 C. During egg case construction, the female is quite focused on her task and not easily induced to abandon it, even in the face of repeated disturbance. Immediately after the egg case is completely constructed, however, the female does abandon her creation (Fig. 3L). Dissection of completed cases revealed that the egg stalk remains attached to the egg (or to the external layer that may surround it) but not necessarily to the case itself. Small holes were often observed in the case apex of newly emerged ‘hatchling’ larvae, revealing where the stalk passed through the case.

In our studies, *N. platani* egg cases were initially green (Fig. 4F), but quickly hardened and turned dark brown (Fig. 4G). Egg case color also varies among, and to varying degrees within, *Neochlamisus* taxa, ranging from very pale tan through extremely blackish-brown, and sometimes appearing reddish, especially in the willow host form. Egg size likewise varies, although lengths of 1.5-2 mm and widths of 0.6-1.1 mm are typical (LeSage 1984a). *Neochlamisus* egg cases are generally smoother than those of other Camptosomata (LeSage 1984b, 1986, Erber 1988), but sometimes possess longitudinal ridges that may aid the female in turning the egg (Karren 1964, Erber 1988). These ridges are a part of each plate and vary depending on the orientation of the sclerites in the female's rectum during their production (see Erber 1988 for more details). Females placed individually in bags tied around the branches of plants in a greenhouse sometimes lay over 100 eggs over a reproductive lifespan of several weeks (Janson and Funk, in review), although production of a few to several dozen eggs is more typical for the alder, birch, maple, and willow host forms that have thus far been studied in this regard (e.g., Funk 1998). In the lab, ovipositing females sometimes abandon eggs before they are fully encased. Such exposed eggs wither or succumb to mold and have not been observed to produce larvae.

Field observations. In nature, eggs are laid on the host plant, mostly on leaves, sometimes on stems or petioles, and occasionally on fruits. The particular placement of eggs varies across taxa (Table 1). Generally, only a single egg is deposited on a particular leaf, although multiple yet generally non-clustered eggs are sometimes observed on single leaves when beetles are at higher densities. These patterns indicate that *Neochlamisus* females generally move, at least to different leaves, in between oviposition events. Whereas adult beetles ordinarily fall from their plant when disturbed, ovipositing females (as in the lab assays) will not, making them much easier to capture. Occasionally, clear oviposition ‘mistakes’ are observed, as evidenced by eggs on clearly non-host plants (e.g., monocots) that are adjacent to or entwined with normal host plants.

Larval biology

Emergence from egg case. It is not known and we did not determine how long after oviposition a larva hatches from its egg within its egg case. We did observe that *Neochlamisus* larvae generally ‘emerge’ from their egg case one to two weeks after oviposition, which agrees with emergence times reported by previous authors (Wood 1966, LeSage 1984a, Neal 1989). To accomplish this, larvae first cut away the egg case roof with their mouthparts (Fig. 4H). The absence of case detritus within dishes of emerging larvae hints that larvae may consume this roof material. Next, larvae free their case from the tether-like egg stalk that attaches it to the host plant. This process was observed, for example, in a *N. bimaculatus* larva that extended its body partway out of its case and used its long legs and tarsal claws to contact and pull at the host substrate until the stalk broke, freeing the case. These ‘hatchling’ larvae are then able to crawl about with their head and legs projecting from the case opening while the soft abdomen is completely covered by the inverted egg case, which is carried perpendicularly to the substrate (Figs. 1 and 4J). Larvae of all ages will, if disturbed, often pull their cases down flush with the substrate, restricting access to the animal inside (Wallace 1970, Lawson 1976).

Larval development. Our assays of larval development confirmed previous findings that the period from larval emergence from the egg case to larval formation of the pupal case lasts approximately four to five weeks (Brown 1943, Wood 1966, Neal 1989) at 24 C, with most growth occurring during the final stadium (Fig. 5). These assays also confirmed that *Neochlamisus* possess four larval instars (see also LeSage 1984a, Neal 1989), rather than the three that is the norm for leaf beetles. We consistently

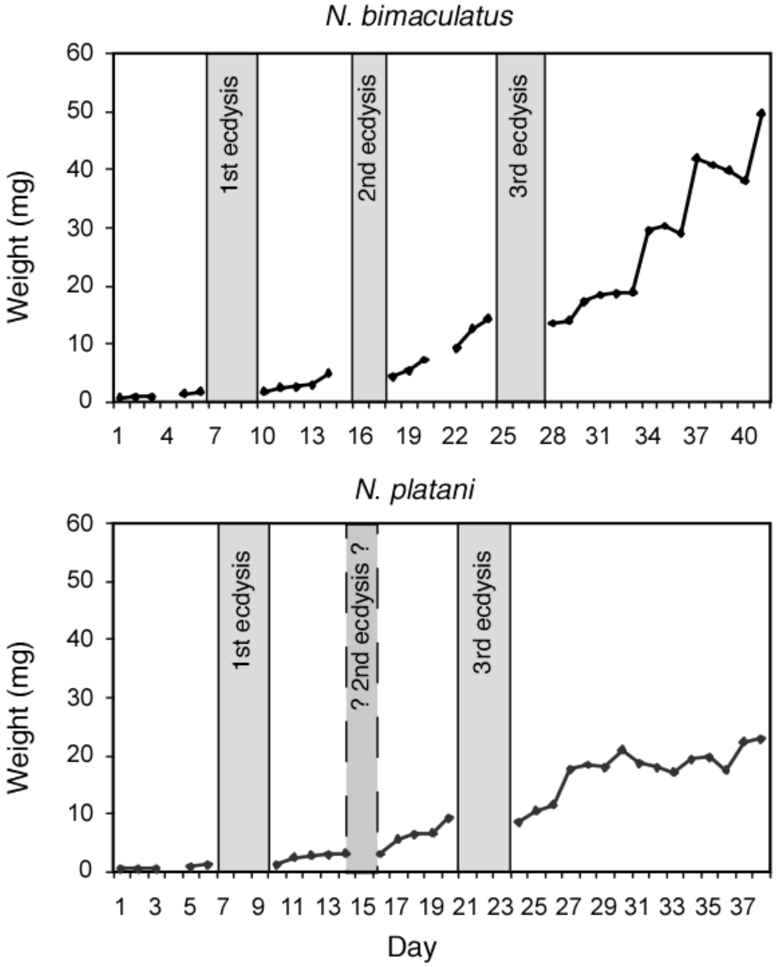

Figure 2-5. *Neochlamisus* larval development. Data are presented for one individual of each of two species. Presented are (with a few exceptions) daily weights from larval emergence until the sealing of the pupal case. Gray bars indicate dates when the larval case was sealed to the substrate, indicating ecdysis-associated activities. The second ecdysis was not actually observed in the *N. platani* individual. This is presumably because of a failure to collect data around day 15: note the constant weight between surrounding dates.

observed that immediately prior to ecdysis a *Neochlamisus* larva cements the circumference of its case opening to the substrate with fecal material. The larva then remains within the cemented case for about three days, during which time it inverts its body (so that the abdomen is near the case opening) and molts (Fig. 4L). After its new cuticle has hardened, the larva frees the case from the substrate, leaving behind a telltale ring of fecal matter and, often, the sclerotized portions of its exuvium (Fig. 4M and N). Interestingly, mouthparts were sometimes found embedded in the substrate within the fecal ring, suggesting that the larva may have used them to provide leverage in escaping its previous skin. The absence of unsclerotized portions of the exuvium hints that the larva may consume them. LeSage (1982) reports that exuviae are sealed into the inner apex of *Exema* cases, but we found no exuviae in *Neochlamisus* larval cases.

Larvae that are physically removed from their cases are capable of continuing normal development if they successfully reenter the case. They are, however, incapable of building cases *de novo*. Furthermore, although more mature larvae sometimes survive for as long as a few weeks under benign lab conditions without a case, these animals rarely pupate successfully and often appear to die of desiccation. First instar larvae are relatively small compared to the case opening and must be carefully handled to avoid separating them from their cases. They are readily capable of reentering their cases, however, and seem to actively seek them out (as in Briggs 1905). By contrast, older larvae possess abdomens that are wider than the case opening and these larvae are less readily removed from the case and less mobile and capable of re-entering the case if removed from it. These differences may reflect different selection pressures across larval stages. For example, a hatchling larva that is struggling to pull its case free from the substrate may accidentally pull itself away from the case in the process, but have a good chance of re-entering the case so long as the case remains attached to the substrate via the egg stalk. By contrast, a case abandoned by a mature larva will surely roll off the leaf and be lost, condemning the larva to death. These two situations might select for an increased capacity to re-enter the case in the former situation, and a decreased capacity to leave the case in the latter.

Case construction. Because *Neochlamisus* larvae are not physically attached to their cases, they possess the maneuverability necessary for case construction (see below). An *N. eubati* larva, for example, was observed deep inside the apical portion of its case while enlarging it. This larva's case remained upright on a leaf, to which it appeared to be loosely attached with a white stringy substance. Such adhesion may allow the larva to release the host plant and continue case construction activity without falling off the leaf.

Neochlamisus larvae are also superbly shaped for fecal case construction, with abdomens that are bent within the case such that the anus is in reach of the mouthparts (LeSage 1984a) (Figs. 1 and 4I). Larvae do not add fecal matter in the form of discrete plates like their mothers but rather as a paste that is formed into a relatively smooth-walled case. *Neochlamisus eubati* larvae were observed to take fecal matter from the anus with their mouthparts, combine it with a regurgitated yellow fluid – which otherwise hardens quickly and is hereafter referred to as the ‘solidifying secretion’ – then add this mixture to the edge of the case opening like a bricklayer applying mortar to a wall. Like bricks, these cases become extremely hard when completely dry; unlike bricks, the cases become quite pliable when moistened. Willow host form and *N. eubati* larvae were also observed to quickly and completely repair author-inflicted case damage by filling in holes with new material (Fig. 4O). Not all feces are used in case construction, as some are left behind as loose pellets. A fluid that is visually similar to the solidifying secretion is also regurgitated by irritated individuals, perhaps as an anti-predator defense (e.g., Pasteels et al. 1988) much like the hypopharyngeal secretions of various insects.

In regard to the specifics of case construction, cases were enlarged in both length and width to accommodate larval growth. The former was accomplished by the addition of fecal matter in layers parallel to the entire circumference of the case opening (Fig. 2A). More material was added to the dorsal edge of the opening (where the larva's head would normally emerge) than on the ventral edge. In addition to lengthening the case, these uneven additions had the effect of gradually decreasing the angle at which the long dimension of the case was oriented relative to the substrate, from an initial 90 degrees to approximately 45 degrees in the pupal case (Fig. 3B and C). Our color-coding of new fecal material illustrates the frequency of these additions through the multiple stripes seen on the case dorsum (Fig. 2E).

Increasing case width requires a more complicated maneuver. This occurred by the mandibular bisection of the case along nearly the full length of its ventral side, the spreading apart of the case at this suture, and the filling in of the resulting triangular gap with new fecal material (Fig. 2B). The precision of this "ventral suture" formation was revealed by the painted cases, which demonstrated, for each fecal addition, the exact splitting of the previously added material into two even halves, with new material in between. This is illustrated by the longitudinal stripes along the venter of a pupal case (Fig. 2G). In some *Neochlamisus* taxa (particularly the alder host form and *N. chamaedaphnes*), lines that illustrate patterns of fecal addition are also visible in natural cases as brown stripes in alternating shades, perhaps reflecting feeding on different host plant tissues over time (e.g. Fig. 4Q). In some other taxa (e.g., *N. comptoniae*), case color is homogeneous and no stripes are visible. In dissections of *N. bimaculatus* pupal cases, the ventral suture appeared as a rough trench along the interior wall, which is otherwise extremely smooth. The case wall is of approximately even thickness across its circumference in cross section, except for a slight indentation at the ventral suture, illustrating the thinner, more delicate nature of this structure. This suture is the first place the case breaks under pressure, especially when the case is moist.

The fate of the original egg case material varies, depending on whether the suture is cut deeply enough toward the case apex to bisect it or only bisects material that has been added following larval emergence. In the former situation, this egg case material is represented in our assays by a red-painted patch towards the posterior apex of the pupal case (Fig. 2). In the latter situation, the egg case survives relatively intact as a small "nipple" that extends from the otherwise rounded case apex and points ventrally (Fig. 1C). While this nipple is typical of species of *Neochlamisus*' sister genus, *Exema*, its frequency and prominence varies considerably among *Neochlamisus* taxa. For example, it is a general and obvious feature of *N. platani* cases, but appears to be entirely absent in many taxa, such as *N. eubati*.

Daily observations on case construction by several *Neochlamisus* taxa revealed that these processes begin three to four days after the larva hatches from the egg case and thereafter occurs throughout larval development (Fig. 6). This continuous construction contrasts with Erber's (1988) suggestion that larvae primarily alter the case just prior to a molt. Measuring the amount of material added to the dorsal rim versus the suture over time reveals an initial investment in the lengthening of the case and a primary investment in case widening during the last stadium (Fig. 6). Among the four willow host form individuals that were measured daily, the mean proportion of total fecal material that was added to the ventral suture (= mm added to suture / [mm added to suture + mm added to rim]) prior to the third molt was 0.42 with a range of 0.32 – 0.50, whereas after the third molt these values increased to a mean of 0.69, and a range of 0.65 – 0.73. This late structural shift is critical for making the case sufficiently rounded and capacious to accommodate the stout adult beetle that will ultimately eclose within it.

An assay of five *N. eubati* larvae kept under conditions of constant light showed temporal patterns of case addition that were highly regular, consistently occurring every other day. However, because these ‘building days’ always immediately followed the provision of fresh foliage, these results may simply indicate that casework occurs whenever higher quality or novel foliage is encountered, rather than that these animals possess a highly regular biological rhythm of case building and rest. Nonetheless, not only the regular patterns of activity but also the absolute amounts of fecal addition were highly concordant among the five study animals of this assay, further hinting at possible stereotyped building behaviors that deserve further investigation.

An additional feature of some *Neochlamisus* cases is plant trichomes. Whereas the cases of camptosomates are generally ‘naked’ in appearance, *N. platani* and the birch, maple, and willow host forms of *N. bebbianae* have cases that are often ‘fuzzy’ in appearance due to variable levels of accumulated plant hairs or trichomes on the exterior of the case (Fig. 1C). In the extreme, these trichomes may be so thick as to completely cover the case, so that it resembles a pinkish or yellowish cotton ball (Fig. 4W).

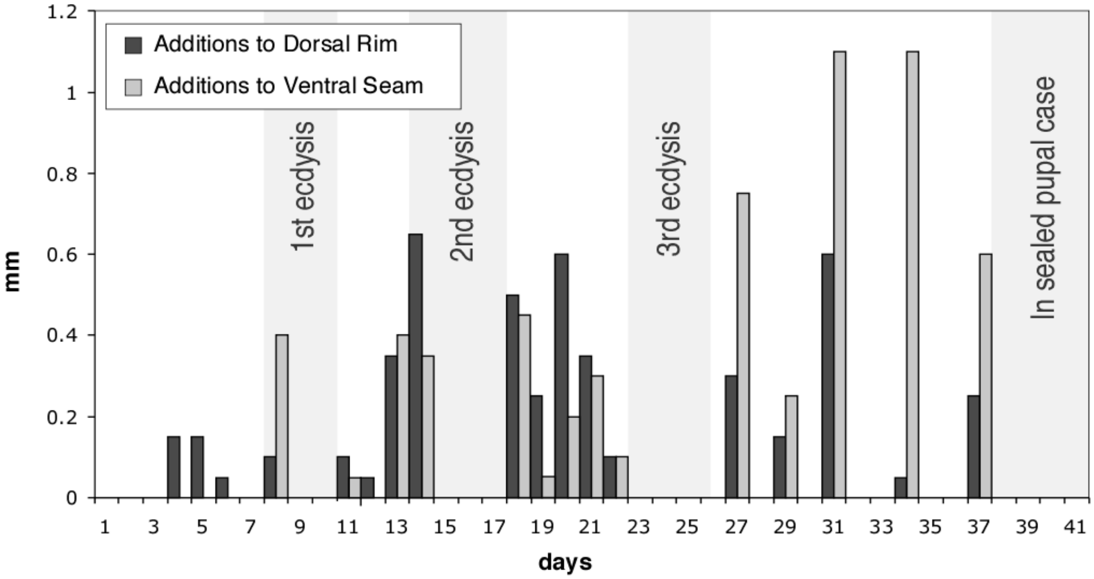

Figure 2-6. Temporal patterns of case construction. Depicted are measurements (in mm) of new fecal material additions to the case of a willow host form individual (see Fig. 2A, B). Measurements were taken each day following the initial painting of the case on the day of larval emergence (day 1). Gray bars indicate dates when the larval case was sealed to the substrate, indicating ecdysis-associated activities. Note the later emphasis on additions to the suture region. This broadens what will ultimately be the pupal case, in preparation for the wide-bodied adult that will develop within it. These data were also collected from three additional individuals; see text for details.

In each of these four taxa, newly emerged larvae migrate towards the youngest leaf tissues of their host plants, which are more highly pubescent than those of other *Neochlamisus* hosts. Trichome incorporation might occur by any of several mechanisms. It could occur passively by the consumption and passage of trichomes directly into the case-building material. It could occur passively as trichomes adhere to moist, newly added fecal material when the larva moves about the plant. It could also be the result of the active placement of trichomes onto the case by the larva.

These issues have not yet been systematically evaluated, but some initial observations are informative. First, when trichomes are particularly thick on the outside of the case, most of them are often easily removed, indicating that they are not integrated into the case material. Second, we observed a first instar *N. platani* larva actively cutting individual trichomes with its mouthparts and discarding them with a backward toss of the head whereupon they became entangled with previously attached trichomes around the case opening. It thus seems quite possible that the already attached trichomes of this individual had themselves been thrown onto the case in the same manner, with the basal layer of trichomes adhering to recently added, moist fecal material. (Interestingly, this trichome-cutting was part of a general clearing of trichomes from a portion of a sycamore leaf, perhaps to expose edible leaf surface for larval feeding.) Third, we repeatedly observed that a single night was sufficient for newly added fecal material on the cases of young willow host form larvae to become completely covered with trichomes (Fig. 4P).

Plants appear to have evolved trichomes partly as physical and chemical defenses against feeding by small herbivores (Goertzen and Small 1993, Valverde et al. 2001, Ranger and Hower 2002, Andres and Connor 2003). Some insects, however, have made use of trichomes for their own protection, as in "trash packets" (Eisner et al. 2002, Medeiros and Moreira 2002). Evaluating potential functions for *Neochlamisus* case-associated trichomes will require further, experimental, analysis. Particularly intriguing in this regard is our discovery that *N. platani* cases conceal a small trichome-filled compartment, a ‘trichome attic,’ that is sealed off within the nippled apex of the mature case (Figs. 4Y and 7). This attic is a somewhat complex structure that is surrounded by a distinct inner wall that is spatially separated from the primary outer wall of the case and may serve as a physical barrier that deters predators.

Field observations. In nature, *Neochlamisus* larvae are often most readily found on the youngest foliage and apical meristems of their host plants. Larvae generally feed on the underside of leaves, but some taxa also feed appreciably on stems (Table 1). They are not gregarious. Larvae are uncommonly seen walking on their host plants, except on very warm days. Rather, they are most frequently encountered with the case pulled down flush with the host substrate.

Pupation and adult emergence

Lab observations. Mature larvae cement their rounded cases (hereafter referred to as pupal cases) to the substrate prior to their pupal molt. This time, however, the entire area of the case opening (rather than just its circumference, as in previous molts) is firmly sealed to the substrate with some combination of fecal material and the ‘solidifying secretion’, making the case quite difficult to dislodge (Fig. 1C). Via the windows cut into these pupal cases, we were able to monitor the remainder of *Neochlamisus* development. These observations provided the following insights: Within one or two days of sealing

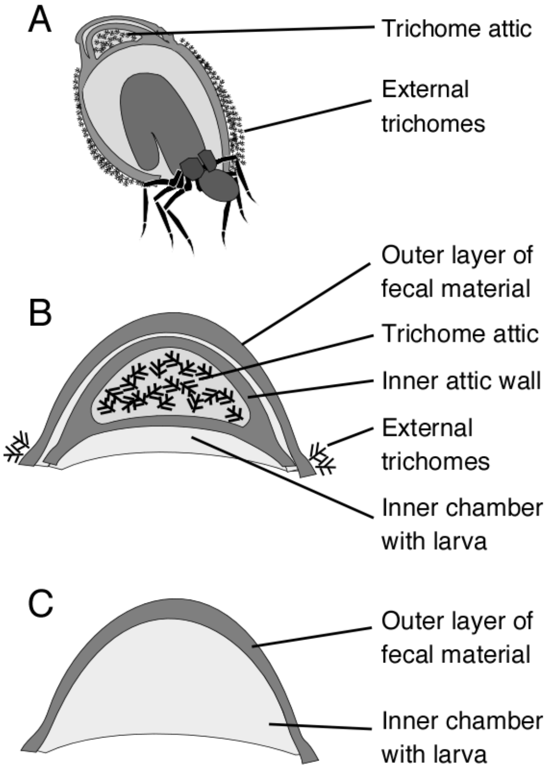

Figure 2-7. The ‘trichome attic’. (A) Location of attic in lateral view of mature *N. platani* case. (B) Dorsal view of *N. platani* case apex, illustrating structure of attic. (C) Absence of attic in *N. comptoniae* case apex.

the pupal case, the mature larva inverted itself and changed from a curved to a straightened prepupal posture, with its head near the rounded apex of the case. On day six or seven following case fixation the larva shed its skin, thus exposing the pupa. This last larval exuvium rested atop the former case opening and was arranged with the head sclerites bordered on either side by those of the three legs, with the mandibles and tarsal claws pointing toward the ventral suture (Fig. 1C). The remainder of the cuticle rested on top of these, underneath the posterior of the pupa. Pupae fit fairly snugly in these cases (Fig. 4S), but are able to rotate within them. Between days 14 and 17, the pupal skin was shed, exposing a soft-bodied, unpigmented, teneral adult (Fig. 4T). The adult assumed its mature pigmentation in less than 24 h (Fig. 4U), but required an additional two to four days for its cuticle to fully harden.

Within a day or two of hardening, the beetle cuts through the side of the case with its mandibles, at a point a couple of millimeters below the case apex (Fig. 4V). Typically starting the incision at the ventral suture, the beetle makes a perfectly even incision around the circumference of the case (Fig. 4W and X), yielding a newly formed ‘cap’. The adult eventually pushes this cap up with its head and legs, crawls out of the case, and abandons it (Fig. 4X). Sometimes, however, the incision fails to complete the circle, such that a small ‘hinge’ is left that still connects cap to case. This cap may remain attached after the case is abandoned, but adults sometimes make a specific second effort to sever the hinge before leaving. Once cutting has been initiated, separation of the cap and adult emergence may occur in as little as 10 m or after as long as several days. Adults appear to be very cautious during this process and cease their cutting for an extended period in response to any disturbance. In the lab, adults that have removed the cap sometimes fail to free themselves from the case, presumably because of the tight fit. When pupal cases are maintained under overly dry conditions, they harden to a degree that apparently prevents the beetles from cutting through the case before they starve. Conversely, pupal cases maintained under overly moist conditions yield high pupal mortality due to mold. These observations may illustrate one fitness tradeoff associated with the case-dwelling habit.

Field observations. *Neochlamisus* generally fix their pupal cases to various parts of the host plant (Table 1). However, mature larvae sometimes stray from their native hosts just prior to the formation of the pupal case, and these cases can sometimes be found on clear non-host taxa that are near the original host plant (Fig. 4Q) (Brown 1943, Neal 1989). The function of this wandering is unknown. It sometimes appears that individuals of certain taxa may preferentially attach their pupal cases where they will be most cryptic, for example, in the position of leaf buds or the forks of similarly colored branches (Fig. 4R), although this hypothesis has not been carefully evaluated. A more clearly documented behavior is a tendency of pupal-case-forming individuals to aggregate to a degree that cannot be explained by beetle densities and the availability of potential pupation sites. It is not uncommon to find one pupal case fixed to another at a site where it takes a few minutes of focused searching of considerable foliage to find a single animal. In the extreme, an interconnected cluster of 12 pupae was found at a high-density alder host form site. These patterns are also observed in the lab, where larvae will form such clusters if reared together. Intriguingly, adults of the newly emerged generation are sometimes seen resting on as yet unopened pupal cases, although these observations are anecdotal. The causes and biological significance of these case-associated behaviors await rigorous study.

Adult biology and phenology

Following emergence, new generation adults spend a few months feeding and mating on their host plant before undergoing an apparently obligate winter reproductive diapause (Janson and Funk, in review) in unknown locations (Fig. 8). Upon emergence from this diapause in the spring, adults resume host-associated feeding and mating, and females initiate oviposition. Adults are diurnally active and most readily collected on warm sunny days, when they often rest on host plant surfaces that are exposed to the sun. On hot days, adults may fly if disturbed, but will otherwise withdraw their appendages into ventral grooves and roll off the plant. During cloudy periods they retreat to more protected positions, close to the stem or within folded or dried leaves, where they often spend the night.

Comparisons with other camptosomates

All camptosomate taxa appear to comprise a monophyletic leaf beetle lineage (Farrell 1998, Duckett et al. 2003) that originated in the late Cretaceous (Farrell 1998). A parsimonious interpretation of these findings suggests that the ancient case-bearing habit has a single origin, representing a synapomorphy of the Camptosomata. Accordingly, the larvae of camptosomate taxa also share a soft curved abdomen that offers little protection from predators or desiccation, but allows ready access to building materials. They further have relatively long legs compared to typical chrysomelids, as these must be extended

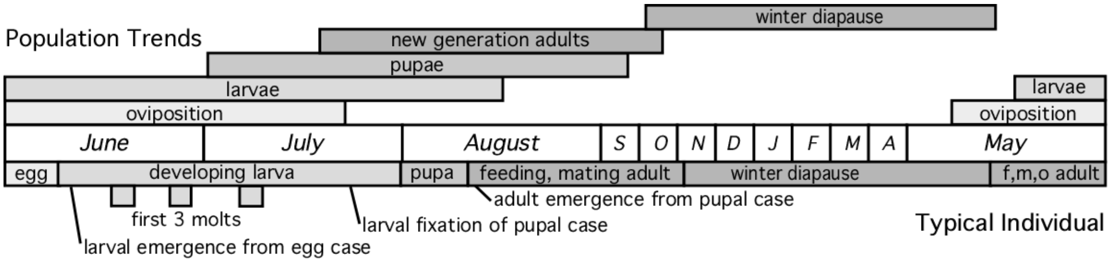

Figure 2-8. Temporal aspects of *Neochlamisus* life history. This figure depicts patterns typical of the development of an individual beetle (below calendar) and of population-level activity in the field (above), based on lab and field observations. Seasonal timing varies according to latitude and altitude and (to a degree) taxon, but that depicted here is typical of the maple host form in Long Island, New York. F = feeding; m = mating; o = ovipositing.

through the case opening. With respect to case structure and associated behaviors, some of these have differentiated along with camptosomate taxa over the last several dozen million years, while others have remained strikingly consistent. Here, we compare our findings on these latter issues with what is known from other camptosomates and especially with Erber's studies on individual cryptocephaline, clytrine, and lamprosomatine species, and with various workers' investigations on *Exema*, the chlamisine sister genus of *Neochlamisus* (Funk 1999).

Most cryptocephaline and clytrine larvae live in and feed on leaf litter (LeSage 1985, 1986, Erber 1988, Stiefel 1993) and some clytrines live in ant nests, feeding on dried foliage or, reportedly, their ant hosts (Riley 1882). Little is known about lamprosomatine larval feeding, but some are known to eat dry leaves (Erber 1988). Various camptosomates are also thought to feed on mold, bacteria, or fungi in the leaf litter (Reid 1999, Slosser 2003). Most chlamisines appear to feed on living plants, but are more polyphagous than *Neochlamisus* (Karren 1966). Most species of *Neochlamisus*, by contrast, primarily use a single plant genus or species as hosts and it appears that their larvae rarely move from their natal plant. These larvae are incapable of survival in the absence of their host plants (Brown 1943, Karren 1972) and generally exhibit suboptimal development and elevated or complete mortality even when maintained on the hosts of their congeners (Brown 1943, Karren 1972, Funk 1998).

Much of the variation in camptosomate case-associated biology is likely due to this ecological variation among taxa. For instance, some ground-dwelling taxa do not produce a stalk (Fig. 1) that attaches their eggs to a specific substrate. Instead, these females drop completed egg cases onto the ground (Erber 1988). Egg stalks are found within the clytrine, chlamisine, and lamprosomatine groups, however. Some ground-dwelling species are also known to withdraw their body and legs into their cases when disturbed so that the case falls on its side and rests on the ground (Kasap and Crowson 1976, Lawson 1976). In this position the flattened and heavily sclerotized head capsules of these animals are used to effectively seal the case opening against predators, such as ants (Erber 1968, LeSage 1985, Stiefel 1993). By contrast, leaf-dwelling taxa, and especially specialized shrub- and tree-associated species such as *Neochlamisus*, would likely perish if they adopted this strategy, as they would fall to the ground with little chance of finding their host again. Rather than releasing the substrate and withdrawing into the case upon disturbance, these larvae cling more tightly to their host and pull the case down over them and flush with the substrate to prevent access (Fig. 1B) (Wallace 1970). Assisting this behavior are tarsal claws that appear more curved than those of ground-dwelling taxa (Root and Messina 1983, LeSage 1984a, 1985, 1986). Leaf-dwelling camptosomates also have more rounded larval head capsules (LeSage 1982, 1984a) that are not used as case ‘plugs’.

In all camptosomate taxa studied, ovipositing females hold the egg against the fovea with the hind tarsi, initiate the case from the egg’s posterior apex, and then extend it along the longitudinal axis (as in Fig. 3) (Erber 1988). There is no previous mention of the role we observed for abdominal bristles in fecal plate formation, but Erber provides detailed descriptions of the fecal plate-forming rectal sclerites and muscles of the four major camptosomate taxa. Females in most previously studied taxa construct the egg case in a manner similar to that described here. The durations of plate and egg case construction also appear to be taxonomically homogeneous (Lawson 1976, Root and

Messina 1983, Erber 1988). However, there is much taxonomic variation in plate shape, external case surface patterns, and egg case-making materials used. Erber (1988) mentions taxa that cover their eggs using only feces, using only an anal secretion, or with wood fibers. Slosser (2003) reports that the clytrine *Coleothorpa dominicana francisana* wraps each egg, but deposits these in clusters on cactus spines with a "funnel" of fecal material surrounding the spine basal to the cluster. The chlamisine *Fulcidax monstrosa* apparently does not use plates at all, but rather wraps its eggs in a long strip of fecal material (Flinte and de Macedo 2004). Egg cases, therefore, vary greatly in structure and overall appearance and provide valuable characters for identifying taxa.

Erber reports that other camptosomate larvae break through the top of the egg case by cutting and then pushing off a “round lid”. No such lid has been reported for *Neochlamisus;* rather, larvae have been observed to first cut a small hole in the center of the case roof before breaking up or devouring the rest of it (Fig. 4H). Erber’s study species do not construct egg stalks, but Karren (1966) reports observations of *Exema* larvae initially detaching their egg cases from the oviposition site by exiting the case, crawling along it, and severing the stalk before re-entering their new larval case. By contrast, we report observing that *Neochlamisus* pulls its egg case free while within it. Indeed, observations of thousands of *Neochlamisus* eggs and first instar larvae in the field have never revealed the behavior reported in *Exema*. Karren (1972) also mentions *Neochlamisus* cutting the egg stalk from inside the case (see also Messina and Root 1980), but we did not examine this possibility. Both of the case-freeing mechanisms described by Karren would require that larvae simultaneously hold on to the egg case and the leaf in order to prevent the larva and/or case from falling from the host.

All camptosomate larvae develop within their cases and continue to enlarge them, but this process also varies across taxa (Erber 1988). Despite the similar morphologies of the larvae, their cases are strikingly varied (Monros 1952, Erber 1988, Flinte and de Macedo 2004). A number of studies describe the addition of fecal material to the rim of the case opening, but there is surprisingly little mention of the ventral suture that allows the widening of *Neochlamisus* cases (but see Briggs 1905, Erber 1988). Nonetheless, a pattern of fecal additions similar to those by *Neochlamisus* is apparent in the cases of some taxa (LeSage 1984b, 1985, Erber 1988), though not others (LeSage 1982, 1986, Erber 1988). Although *Exema* has been studied by several workers, there is no specific mention of its forming a ventral suture or adding new material to anything but the rim of its case (Karren 1964, 1972, LeSage 1982, Root and Messina 1983). *Exema* adults and cases both tend to be more elongated than those of *Neochlamisus*; however, their cases clearly do increase in width during larval development. Consistent with the possible lack of a suture, however, is the fact that the original egg case in *Exema* is never cut into, so that their cases always retain a distinctive nipple that is often noticeably lighter-colored than the rest of the case (Brown 1943). LeSage (1982) reports that this nipple consists of various compartments in which the shed exuviae are sealed via fecal material. This procedure resembles that used by nipple-cased *N. platani* larvae to form their trichome-filled “attics”, raising interesting questions about the function and evolutionary history of these structures and behaviors.

Various casebearers are reported to incorporate quite a diversity of materials in their larval cases including wood fibers, paper, sand, soil, and even artificial substrate (Karren 1964, Erber 1988, Slosser 2003). By contrast, we have only known

Neochlamisus to employ fecal material and trichomes in case construction, although Karren (1972) reported "other debris" being used in their cases. Karren (1964) also reports trichome incorporation in one species of *Exema*, *E. pennsylvanica*. As we have also observed for *Neochlamisus*, the cases of *Exema* are attached to the substrate during molts partly by a material that appears as thin white threads when the case is moved (Boldt and White 1992).

Here we present the most detailed report of the temporal and spatial patterns of case additions and enlargement for any camptosomate. We show that larvae add to their cases throughout their juvenile life, and possibly in a conserved rhythmic pattern. Unlike the taxa studied by Erber, *Neochlamisus* does not restrict its case construction to periods immediately prior to molting and does not seal the entire area of the case opening to the substrate during the first three larval molts, instead sealing only the opening’s perimeter. Like other taxa, however, *Neochlamisus* does seal the entire opening before pupating. The time required for larval development is rather similar to that of some other camptosomate taxa (LeSage 1982, 1984a, b, Stiefel 1993), although other camptomsomates undergo long periods of dormancy inside the case. For instance, many of the myrmecophilous species overwinter as larvae (Erber 1969), and some taxa undergo diapause in sealed cases before or after pupation (LeSage 1984b, 1985, Slosser 2003, Flinte and de Macedo 2004). In *Neochlamisus*, however, only adult winter diapause is known. Interestingly, all studied Camptosomata appear to leave their pupal case by the cap-cutting approach.

The present study reports diverse aspects of *Neochlamisus* behavior, life history, and case architecture for the first time. It further summarizes and embeds what is known about this genus within the broader context of camptosomate diversity and evolution. Our findings specifically point towards future studies on many elements of the biology of these intriguing case-dwelling insects.

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CHAPTER III

TESTING THE EFFECTS OF FECAL CASES ON SURVIVAL AND GROWTH RATE OF LARVAL *NEOCHLAMISUS PLATANI* IN FIELD CONDITIONS

Abstract

Camptosomate leaf beetles build and carry a case of fecal material as immatures. Fecal cases are known to protect larvae and pupae from predators and to interact with abiotic conditions, such as temperature and humidity, in ways that both benefit and reduce fitness. However, whether fecal cases are essential for survival per se is seldom tested and usually only in laboratory conditions. Our study seeks to determine the relative impact of fecal cases on predation, parasitism, and growth rate in a field setting. We do so using cased eggs and cased and caseless larvae of *Neochlamisus platani* reared on sycamore saplings either with a predator-exclusion bag or open to predation. Third instar larvae can develop to pupation successfully without cases in benign lab conditions, but we found that fecal cases are required for *N. platani* survival in the field. Individuals placed on trees as first instar larvae without cases did not survive more than five days, the vast majority dying within two. We found no effect of the bags on survival, so predators were not the main cause of larval death. Instead, caseless larvae may be less able to cope with abiotic conditions. Development time in the field matches closely with growth rate in the lab. Since predation of *Neochlamisus* is poorly documented, as is arthropod community structure on sycamores, we also provide a list of potential predators and competitors observed in the field in the course of these studies.

Introduction

Herbivorous insects are often highly susceptible to attack from predators because of their habit of feeding on exposed leaf surfaces (Bernays 1997). To avoid being eaten, herbivorous insects have evolved a myriad of defensive behaviors and morphologies. Animal architectures, structures built by an animal individual or group, can prevent predators from finding or reaching prey. Nests, burrows, and cocoons, for example, are intricately constructed to protect the builder or its young from predators and other environmental factors (Hansell 2005).

Perhaps one of the most bizarre adaptations to prevent attack is the construction behavior of the camptosomate leaf beetles, which use their own feces to build a case in which juveniles live. The Camptosomata are a group of two subfamilies of leaf beetles that share the common trait of casebearing. Immediately after laying each egg, the adult female covers it in plates of fecal material, creating a fecal case (Erber 1988, Brown and Funk 2005) (Fig. 1A). After hatching from the egg itself, each larva chews open one end of its egg case, but does not abandon it. Instead, it carries the case and enlarges and adds to it throughout the juvenile stadia (Fig. 1B). Eventually the case opening is sealed and pupation takes place inside. Several possible functions for fecal cases have been proposed, but usually consist of preventing predation and desiccation. Although few predation events of camptosomates have been observed in the wild (Erber 1988,

Figure 3-1. Experimental treatments and field setup. (A) *Neochlamisus platani* egg (inside case) on *Platanus occidentalis* leaf. (B) Cased larva. (C) Caseless larva. (D) Predator exclusion treatments: (left) sycamore tree with a bag and (right) tree with no bag. (E) Experimental setup: line of 20 pools, each with 6 trees (3 with bag, 3 no bag).

Olmstead 1994, Cox 1996, DJ Funk personal observation), pupal cases are sometimes found empty and torn open while still attached to the host plant (Flinte and Macédo 2004, DJ Funk personal observation). The larvae of the casebearing beetles are conspicuously orange, soft-bodied, and vulnerable when the case is removed (Chapter IV). No one has investigated the chemical defenses of these larvae, but laboratory studies show that arthropod predators are not readily deterred by caseless larvae (Root and Messina 1983, Flinte and Macédo 2004, Chapter IV). These studies show that fecal cases, however, reduce predation by creating a barrier to attackers. These attackers have included ants (Wallace 1970), hemipterans (Root and Messina 1983, Chapter IV), omnivorous orthopterans (Chapter IV), and spiders (Chapter IV). Fecal cases may also serve to camouflage larvae, preventing attack altogether, but this has never been experimentally tested.

Casebearers often face intense pressure from parasitoid wasps, which have been observed to attack as much as 90% of eggs (DJ Funk unpublished data) and similar amounts of larvae (Erber 1988). Fecal cases could nonetheless reduce the diversity or number of successful parasitoid attacks, since some specialization is needed to bypass the case. Parasitoids of the casebearing beetles must penetrate the completely encapsulating egg cases (personal observations), but can attack larvae through the case opening where the head and legs emerge (Schöller 1999). Still, several types of wasps are known to attack both eggs and larvae of casebearers (Erber 1988), and structures made of feces, although protective against predators, can actually attract parasitoids (Müller and Hilker 1999, Schaffner and Müller 2001, Weiss 2006). Interestingly, once successful, the parasitoid's offspring benefit not only from an ample food supply, but also from the anti-predator protection of the case. Camptosomates may have evolved fecal cases as a way of deterring predator attacks, but may now suffer increased conspicuousness to parasitoids as a consequence.

So far, the few tests of fecal case function have taken place in artificial laboratory settings, consisting of small arenas and hand-selected predators. Here, we investigate the effects of the case on survival of young casebearing beetles using predator-exclusion bags in a controlled field experiment. This setting exposes larvae to all environmental conditions, including all potential predators and abiotic factors. It also allows any anti-predatory functions of the case to act at all levels including primary (i.e., camouflage) and secondary (i.e., physical barrier) defenses. In addition, we compare temporal development patterns from this test with that found in the laboratory (as presented in Brown and Funk 2005). This experiment was designed to test questions concerning the ecological interactions of a defensive structure and its surroundings, such as: 1) What are the relative selection pressures larvae face from parasitoids and predators? 2) How well do fecal cases reduce the impact of these pressures? 3) Can caseless larvae survive in the field, even though they have difficulty doing so in the lab? 4) Are larvae without cases, but sheltered from predators, freed from the burden of carrying and maintaining a case and thus develop in less time? 5) In the absence of predation, do caseless larvae suffer more from the negative effects of abiotic factors? We test these questions using eggs and larvae of *Neochlamisus platani*, the sycamore leaf beetle.

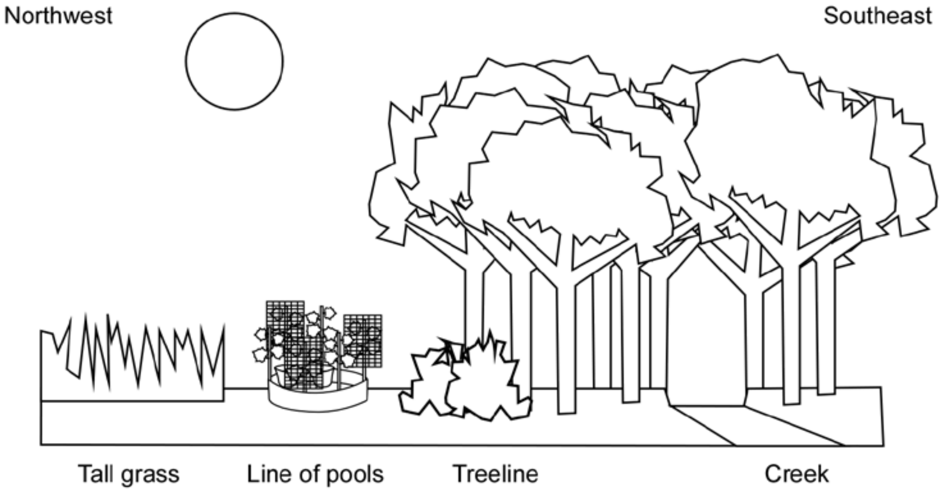

Figure 3-2. Diagrammatic representation of the field setup as shown perpendicular to the line of pools and treeline. Figure not to scale.

Methods

Test animals

N. platani are small univoltine leaf beetles that feed, mate, and oviposit exclusively on American sycamore, *Platanus occidentalis*. Eggs are laid in the spring and larvae feed through spring and some of the summer. Adults emerge from their pupal cases in the summer, feed and mate, and overwinter until spring. Both the host and beetles can be found over most of the eastern half of the United States in riparian and disturbed habitats (Karren 1972). They can also be found in urban settings if host plant quality is appropriate (personal observations).

Eggs: Adults were collected in and around Nashville, TN during the spring of 2007 and allowed to oviposit on leaves in the lab. The resulting eggs were removed, along with a circa 1cm square of the host plant so as not to disturb the egg stem which attaches eggs to the leaf surface (Fig. 1A). Leaf squares containing an egg were attached to stems and leaves of plants in the field using wood glue (Elmer's, Columbus, OH). Most eggs were placed on the abaxial side of leaves, simulating their location in the wild (Brown and Funk 2005). Any larva hatching from an egg in the field should naturally maintain its case.

Larvae: Eggs collected near where this study takes place were maintained en masse in 5 cm Petri dishes in an incubator at 24 C and 14:10 light:dark cycle and checked daily for hatchling larvae. Newly hatched larvae were then divided into two treatments: 1) cased larvae , i.e. unaltered larvae with cases (Fig. 1B) and 2) caseless larvae, i.e., larvae whose cases were removed with forceps (Fig. 1C). Larvae are not physically attached to their cases and are not injured when these are removed. Caseless larvae will not create another case, but are sometimes able to develop to adulthood without one in the laboratory, at least if it is removed from a late-instar larva (personal observation).

Host plants

Small sycamore trees were bought in early spring as bareroot plants, or with leaves in 4L pots from a local nursery and maintained in the greenhouse at Vanderbilt University until all leafed out. They were then placed in an old field next to a wooded area containing mostly hardwoods (including *P. occidentalis*) surrounding a small creek (Fig. 2) at Ellington Agricultural Center in south Nashville. Since *N. platani* and sycamores have been found near this site over multiple years, it is considered natural habitat for both. Experimental *P. occidentalis* trees were placed in 180 cm plastic "kiddie" pools that were filled with water as needed and arranged in a line parallel to and within 3m from the treeline. Each pool contained six trees, trimmed to four leaves each to equalize leaf biomass, and arranged so that their branches did not touch (Fig. 1D,C).

Predator exclusion treatments

Eggs, cased larvae, and caseless larvae were each divided evenly into two treatments that differ according to exposure: 1) covered by a plastic bread bag with holes small enough to exclude both predators and parasitoids, but which allowed air and water exchange, and 2) no bag and therefore exposed to both predators and parasitoids (Fig. 1D). Bags were supported by a wire frame that wrapped around the plant stem below the leaves. The bags were tied tightly around the stem and wire with twist-ties, closing off the foliage and animals inside. Each bag had a 13 cm^2 window cut into one side and a removable door made of clear plastic held with Velcro, permitting access to the inside without removing the bag.

Experimental setup

120 trees were placed in 20 pools (6 trees = treatments/pool). Each tree in a pool ultimately contained five individuals of one of the six treatments (i.e., combinations of egg/larvae, cased/caseless, bag/no bag treatments) (Fig. 3). Trees within a pool were assigned a treatment randomly, but each pool contained a complete replicate of treatments (20 replicates, = pools, in total) (Fig. 1D). Since eggs and larvae were not simultaneously laid/emerged, we could not set up the entire experiment at once. Instead, individuals were added to one tree as they emerged until that tree had 5 individuals. Then we added individuals to another tree within the same pool. This process continued until all trees within the pool had five individuals. Only then were individuals added to a tree in the next pool. The order of setup was completed randomly across treatments within each pool and across pools to reduce biased effects of timing and location in the field. Eggs were added over six days, larvae over 25 days.

Data collection and statistics

The presence/absence of individuals was recorded once every two days following their placement on the plant until they disappeared or began pupation. *Neochlamisus* are very dependent on their host plants for food and although they can be quite mobile within a plant, are very unlikely to leave their host plant willingly (Brown and Funk unpublished

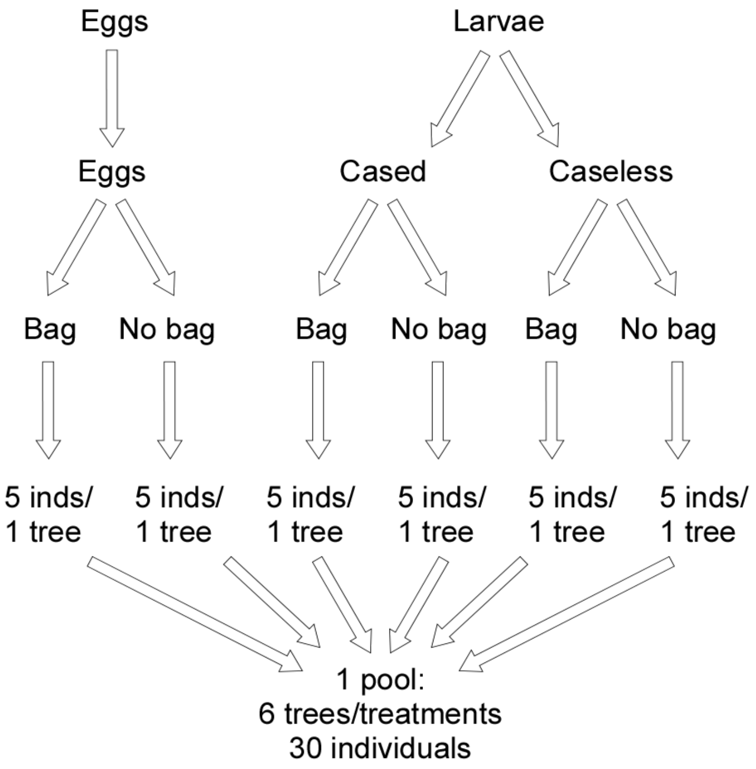

Figure 3-3. Experimental design. Eggs were divided into bag/no bag treatments. Larvae were divided into cased/caseless treatments, which were then divided into bag/no bag treatments. Five individuals were added to each of the 6 trees in a pool, with each tree representing a different treatment. Twenty pools were set up in the field. Therefore, the experiment examined 600 individuals across 120 trees.

data). Any individual not found for four consecutive observations was classified as dead. After 40 days, all surviving larvae had sealed their cases for pupation and all eggs and pupae were brought back to the laboratory to check for parasitism. The number of days from placement in the field to pupation/disappearance is analyzed as "survival ability" using three-factor ANOVA with two experimental factors (case and bag) and pools as randomized blocks (*F*). Since individuals were not individually marked, survival time may vary a day or two from actual values.

Results and Discussion

Effect of treatments on survival

Survival rates were extremely low. Only 11 larvae hatched from eggs (six in bags and five without bags). We do not know why so few eggs survived, especially since the same cohort continued to emerge in the lab. Most likely they died in the egg stage due to abiotic conditions (see below). Individuals that hatched from eggs in the field, however, survived 28 +/- 0.46 days on average, indicating that at least these were robust.

Most individuals placed on trees as larvae survived less than five days, despite the presence/absence of a bag (Fig. 4), indicating that predation was not a major cause of this mortality. Bags did not significantly affect survival time for eggs (with bag: 1.7 d ± 0.74, n = 100, no bag: 1.4 d ± 0.64, n = 100, *F* = 0.10, df = 1, P = 0.92) or larvae (Table 1). There was also no effect of the bag when analyzing survival time only of those larvae that survived more than two days (with bag: 15.15 d ± 1.64, n = 55, no bag: 15.07 d ± 1.37, n = 58, *F* = 0.11, df = 1, P = 0.73).

The presence of fecal cases, however, was a major factor in larval survival (Table 1). Ninety-five percent of caseless larvae outside of bags and 93% of caseless larvae in bags died in less than two days. Many were found shriveled and dead on the leaf surface. No individuals in these treatments survived more than five days. By contrast, for cased individuals, only 54% without bags and 48% in bags survived less than two days. Cased larvae survived significantly longer than caseless larvae (Fig. 4, Table 1) and there was no interaction between the bag and the presence of the case (Table 1). Apparently *N. platani* desperately need their cases for survival as young larvae.

In a similar study of another casebearer, *Fulcidax monstrosa*, a chlamisine leaf beetle from South America, Flinte and Macédo (2004) found comparable results to ours, even though they made less frequent observations and used a much smaller sample size (n = 10 for each treatment). Sixty percent of cased larvae without a bag survived at least one month. No individual without a case and without protection from a bag survived more than twelve hours, whereas seven caseless individuals but inside bags managed to survive for four days. These latter individuals were considered "weak" and did not feed. The authors attribute their survival to the bag, which prevented them from falling to the ground and allowed them to reclimb the plant more easily. None was found alive after a month. In our study, only one caseless larva was observed feeding and walking on the leaf surface after two days (on day five), but this was without the aid of a bag. It may have died because it could not rely on the case to remain attached to the leaf during ecdysis (Fig. 5). Despite some survival success of caseless *Neochlamisus* larvae under benign laboratory conditions (Brown and Funk 2005), the current study indicates that larval survival in the field is impossible without fecal cases.

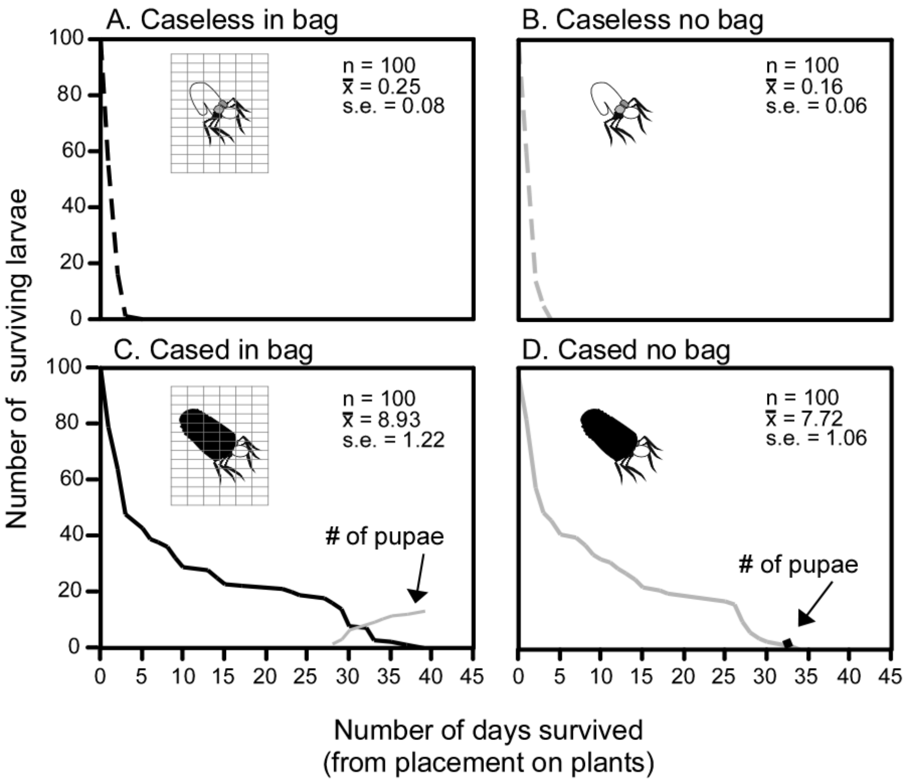

Figure 3-4. Survival time. Number of individuals from each larval treatment surviving over time. Sample sizes, mean survival times, and standard errors are also given for each treatment. The cumulative number of larvae that had sealed their cases for pupation are displayed with separate lines.

Table 3-1. Survival time. Three-factor ANOVA with two experimental factors and pools as randomized blocks.

Source	**MS**	**df**	***F***	**P**
Bag	9.30	1	0.16	0.69
Case	6328.20	1	111.64	<0.0001
Bag x Case	12.60	1	0.22	0.64
Pool (Random effect)	136.22	19	2.40	0.0009
Error	56.69	377	7.17	<0.0001

Rate of development in field conditions

During ecdysis, larvae somehow temporarily attach their fecal cases to the leaf surface (possibly with feces, silk, or some unknown secretion) and shed their skin for the transition between stadia (Brown and Funk 2005). Although we did not intentionally observe ecdysis for fear of disturbing the larvae, there were three periods at which larval cases seemed to be "fixed", as if going through ecdysis, in the field (Fig. 5). Observations centered around 34 days most likely correspond to larvae that have sealed the case in preparation for pupation. Rate of development in the field based on these observations matches measurements recorded for *Neochlamisus* species in the laboratory, in which individuals underwent ecdysis at 7-10, 15-17, and 21-28 days and sealed the case for pupation at ~38 days after emerging from the egg case. (see Fig. 6 in Brown and Funk 2005).

Individuals added as larvae that made it to pupation took an average 32.1 +/- 0.80 days (n = 16) to reach this stage of development. Individuals added as eggs took a little longer, 36.5 +/- 1.5 days (n = 4), but they spent time in the egg case before emerging as larvae. Interestingly, this rate is equal to or less than previous lab results, both for time between oviposition and emergence from the egg case and for time to pupation (Funk 1998, Brown and Funk 2005, Funk et al. unpublished data). Perhaps development time is species specific, since previous studies focus on different, but very closely related species. More likely, the larvae in our study experienced higher average temperatures and higher selectivity of foliage, with less disturbance than those in laboratory conditions, which are typically reared at a constant 24C on leaf clippings in dishes that are replaced

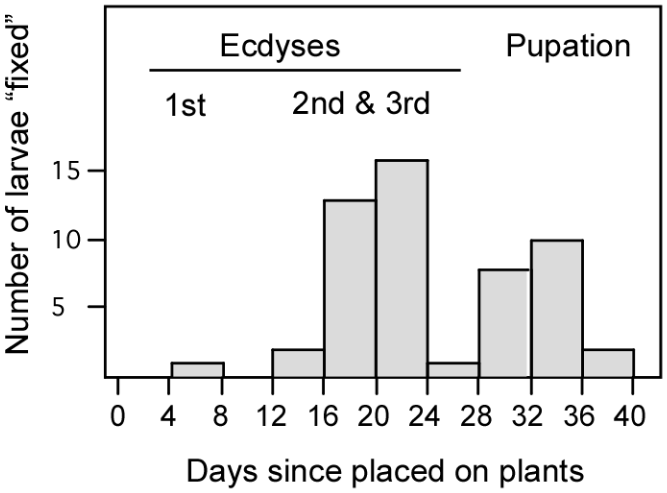

Figure 3-5. Number of observations per day in which larvae were observed to be "fixed" to the substrate as if the larva was undergoing ecdysis. This figure excludes larvae that were placed on plants as eggs, since their timing would be different from those placed in the field as larvae. It also combines larvae in bags and without bags. Categories above the histogram represent which transition larvae are most likely to be undergoing according to data for *N. bebbianae* reported in Brown and Funk (2005).

and cleaned, respectively, every other day. Higher temperatures alone often positively influence growth rate (Kingsolver and Huey 2008).

Twenty pupae were brought back to the laboratory and allowed to complete development in Petri dishes in an incubator set to 24 C. Only five successfully emerged as adults. One adult removed the pupal cap necessary to emerge from its case, but died before escaping. After giving the remaining individuals ample time to develop normally, their pupal cases were dissected. Ten died in the case as pupae and four as fully formed adults that did not emerge from the case. Pupation time (i.e., time between being observed with case sealed and emerging as an adult) averaged 20.5 +/- 7 days, which again corresponds to previously known times (Brown and Funk 2005), but these individuals also spent much of this time in the laboratory under constant temperature. Pupation time in all eastern *Neochlamisus* species studied in the lab and here seems very consistent at around 20 days (Brown and Funk 2005, Funk et al. unpublished data).

Larval wandering

Late-development fourth instar larvae, which have rounded their cases in preparation for pupation (see Brown and Funk 2005), often wandered off the plant. In bags, this resulted in increased observations of fourth instar larvae on the bag rather than the tree. Larvae without bags, however, often disappeared at the time that larvae in bags were sealing their cases. They apparently walked off the plant entirely. One individual was found walking in the dirt of the pot and replaced on the tree, only to be found off the plant again within ten minutes. It had disappeared by the next round of observations. Two fourth instar larvae were found floating in the water surrounding their trees. Another individual sealed its case to material at the base of the tree. We feel that larvae in bags might have walked off the tree too if able. They often chose not to pupate on the plant inside the bags, rather sealing their cases to the bag itself. Apparently there was a strong urge to pupate away from the host. Pupating on alternative hosts and on non-plant substrates has been observed in the field and in the laboratory, respectively (Brown 1943, Neal 1989, Erber 1988, Brown and Funk 2005, *unpublished data*), but it remains much less common than pupating on the host, which usually takes place on branches or leaves. Immediate cues or causes leading them to leave the host are unknown, but might relate to predation risk, humidity, or temperature.

Potential factors contributing to mortality

Predators. Little is known about the natural predators of casebearers (Erber 1988) and we found little evidence of predation in this study, such as observations of larvae being killed or of broken cases. Two large larvae (fourth instar) were possibly killed by a jumping spider (Salticidae) that managed to get into one of the bags. However, most larvae that disappeared did so without known cause. This might be expected given the relatively brief time spent making observations and the immediate mortality of caseless larvae. Since bags did not affect survival, it seems that predation was not an important source of mortality for first instar larvae in our study. However, many predacious arthropods were observed on the trees during the experiment and we list them here as a guide to future investigations.

Potential predators observed on or around trees during this experiment include wasps, bees, dragonflies and damselflies, assassin bugs, spiders, tree frogs, and several

Passerine birds. Insects and spiders on the plants were photographed when possible, aiding identification. *Polistes carolina/perplexus* paper wasps were common visitors to the trees. Similar vespids have been shown to be predominant predators of other fecal-carrying leaf beetles (Schenk and Bacher 2002). Thread-waisted wasps (Sphecidae: *Ammophila nigricans*) were also seen visiting. These wasps typically specialize on caterpillars and so may not be a threat to *Neochlamisus*. However, Scullen (1965) reports finding 55 *Neochlamisus* adults provisioning a nest of *Cerceris fumipennis*, another sphecid wasp. Two predaceous beetles were seen on trees: the spotted ladybeetle (*Coleomegilla maculata*) and the introduced Asian ladybird beetle (*Harmonia axyridis*). These typically feed on aphids, but one coccinelid's larvae were capable of eating *Neochlamisus* larvae with cases in the laboratory (Egan personal communication). Several spiders were seen on and around plants, including a long-jawed orb weaver (Tetragnathidae: *Tetragnathus* sp.) and other orb weavers (Araneidae: *Neoscona* sp.), which may be more dangerous to flying adults that are more likely to get trapped in webs. Wolf spiders (Lycosidae: *Parodosa* sp.) were seen on pots and pools and may not readily climb trees, but could attack large larvae as they wander off the host plant before pupation. Perhaps more threatening to the *N. platani* larvae in our study were cobweb spiders (Therididae), jumping spiders (Salticidae), and crab spiders (Thomisidae), which were found on leaves, stems, and in the folds of stipules where *N. platani* regularly feed. Some predators seen in the vicinity of the trees, but which are probably less likely to affect larval *N. platani,* include hawks, a large skink, a common snapping turtle, and a house cat.

Parasitoids. More surprisingly, only two individuals, one larva from an egg and one put on as a cased larva, died attached to the leaf as if parasitized (see Brown and Funk 2005), but parasitism could not be confirmed. None of the eggs or pupae brought back to the lab produced parasitoids. Since parasitism rates are often high in wild-collected populations during months of peak oviposition, we can only guess that the eggs in this study were not parasitized because of the delayed appearance of our eggs in the field, that parasitoids rely on cues from ovipositing mothers, or some other deficit. For instance, egg parasitoids seem to hop rather than fly and may have been excluded by the pools, or the eggs in our study died so quickly that parasitoids were not attracted to our field site. They may require freshly laid, and thus softer, egg cases to successfully oviposit through the case wall. Alternatively, whatever caused the high mortality of the beetle larvae might have also plagued their parasitoids.

Abiotic conditions. Larval mortality was more likely due to abiotic conditions, such as temperature and humidity, than predation. Most caseless larvae were found dead and shriveled on the leaf surface shortly after placement, as if desiccated, rather than missing or mangled over time, as expected if they were attacked. Indeed, Kaufman (2001) found that larval mortality in another fecal-retaining leaf beetle was most often due to abiotic factors when larvae were young, but predation more greatly impacted late-instar larvae. Several authors suggest that fecal structures help reduce the negative effects of temperature fluctuations, low humidity, rainfall, and even UV radiation (Olmstead 1994, Heinrich 1996, Bacher and Luder 2005, Flinte and Macédo 2004, Brown and Funk 2005, Chaboo et al. 2008), yet these factors are seldom tested directly. In this study, removing fecal cases seems to have left larvae especially vulnerable to some environmental factor.

Bacher and Luder (2005) found that larval development was negatively affected by exposure to intense UV radiation in a cassidine leaf beetle, which carries a loose portable shield over the back, yet they found no effect of the fecal shield in moderating the negative effects of UV on development. UV radiation could affect *N. platani* similarly, or since casebearers are mostly hidden under their cases, their bodies may be even less suited to dealing with radiation than the less-concealed cassidines. Even insects well adapted to an arboreal lifestyle may suffer losses during heavy rains as they lose grip on their host plants or suffer physical damage (Mann et al. 1995, Bonhof and Overholt 2001, Naranjo and Ellsworth 2005, Aviles et al. 2007, von Berg et al. 2008). This too could explain the sudden disappearances of some of the larvae in our study.

Many authors attribute larval death of camptosomates in the lab to desiccation (Ellias and LeRoux 1964, Karren 1972, Boldt and White 1992) and insufficient moisture, especially in caseless individuals, could have caused the shriveled appearance of larvae in this experiment. Interestingly, Brown and Funk (Chapter VI) found that caseless pupae are remarkably able to survive long periods of rather low humidity, indicating that humidity may not be the leading cause of caseless larval mortality. Of course, immobile pupae may be more strongly selected to resist desiccation compared to newly hatched larvae. However, low humidity conditions increase case strength beyond what adults can cut through, thus causing mass casualties (Chapter VI) and although normal lab rearing methods were undertaken, something similar may have happened to the adults in this experiment that were found fully developed, but died inside their pupal cases. It may also explain why larvae seemed so eager to leave their hosts before pupating. They may be searching for areas, such as at the base of the tree and/or in the leaf litter, that are more likely to experience higher humidity, hopefully assuring that the case is weak enough to cut the pupal cap when the time comes.

Whatever the exact cause, the current study, as well as Flinte and Macédo (2004), indicate that fecal cases are necessary for larval survival in field conditions, even when predators are excluded.

Possible competitors

Young *Platanus* leaves are typically covered in plant hairs, called trichomes. Many plants have evolved trichomes to prevent herbivory since they deter small arthropods from moving and feeding on the leaf surface (Levin 1973). *Platanus* trichomes are even known to cause respiratory problems in humans (Ross and Mitchell 1974). *N. platani* however, has no problem feeding on densely pubescent leaves (Chapter V) and actually benefit from the trichomes by incorporating them into their cases where they further enhance the anti-predatory function of the case material (Chapter IV). Other herbivores, however, may not be so fortunate and sycamores often have less diverse herbivore populations than other nearby species (Thompson and Solomon 1986, Morewood et al. 2004, Ulyshen and Hanula 2007, personal observations).

During this study however, we observed several herbivores on the plants that may compete with *N. platani* for resources, although many may simply be visitors. Sycamore lacebugs, *Corythucha ciliata* (Tingidae), and caterpillars from three families of lepidoptera, including *Spodoptera ornithogalli* (Noctuidae), *Misogada unicolor*

(Notodontidae), and a geometrid moth larva, appeared to affect the quantity and quality of foliage. In our observations, sycamores were found to house planthoppers including *Acanalonia conica* (Acanaloniidae) and *Metcalfa pruinosa* (Flatidae) and the leafhopper *Penthimia americana* (Cicadellidae). Feeding in these groups could not be determined from the photographs and they were usually not found on leaf surfaces, the main feeding site of *Neochlamisus*. If they are feeding on sycamore, they may not be direct competitors, but could affect the overall health of the plant. There were several species of acridid grasshoppers that visited the sycamores and one bush cricket (Gryllidae: *Phyllopalpus pulchellus*) was found in the grass between pools, but none of these were observed feeding on the plants. We also saw other herbivorous beetles, including scarabs and another chrysomelid (*Brachyphnoea* sp), although they were also not observed to be eating the leaves. The sycamores were occasionally hit by Japanese beetles *Popillia japonica*, which are not native, but were capable of defoliating the plants, and may have a lingering effect for other herbivores in the environment like *N. platani*. One plant was clipped at the stem, removing all of the leaves and thus any larvae left on them. A white-tailed deer was seen foraging on plants adjacent to the row of pools and may have been the culprit. Deer feed on the new growth of a variety of plants and such a large herbivore not only acts as a competitor, but perhaps also as a predator, if they feed indiscriminately (Ohgushi 2005).

Conclusion

This experiment was designed to answer several questions concerning the ecological selective pressures that the casebearer *Neochlamisus platani* faces in the wild.

Our results, however, were not quite what were expected given observations from collections in the field. In summary: 1) The relative selective pressures from parasitoids versus predators cannot be determined since neither was often observed. In fact, from this study we would have to say that the threat from parasitoids is slight, despite high mortality due to parasitoid wasps found previously. In fact, it is clear that predators were not a major cause of mortality since bagged and unbagged treatments suffered identical mortality. Potential predators are apparent in the habitat, however. 2) Removing the fecal cases from 1st instar larvae was detrimental to their survival almost immediately. Only one individual was observed walking and feeding for more than 4 days. Cased larvae established themselves on the plant at nearly twice the rate as caseless larvae 3) Mortality was unexpectedly high in all treatments and it is our belief that this is due to abiotic factors, possibly including high temperatures, UV radiation, and desiccation. Removing the fecal cases of larval *N. platani* makes them even more susceptible. Low humidity may have also reduced adult survival due to harder cases impeding their escape. 4) Because no caseless individuals survived to pupation, we could not evaluate the energetic costs of carrying a case on development time. Measurable stages of development of cased larvae in the field took place in a manner similar to lab results, including time of transition between stadia and pupation duration. The time it took to emerge from the egg case as larvae and the overall time until the case was sealed for pupation in the field was equal to or less than previously measured. We conclude that fecal cases are very important for successful survival of *Neochlamisus platani* in field conditions, but its specific role in fitness, especially in moderating certain abiotic conditions, remains to be tested.

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CHAPTER IV

ANTIPREDATORY PROPERTIES OF A BIZARRE ANIMAL ARCHITECTURE: HOW COMPLEX FAECAL CASES THWART ARTHROPOD ATTACK

Abstract

Animals create a wide variety of structures to deal with abiotic and biotic challenges. The present study evaluates a particularly intriguing structure whose function has never been thoroughly tested. Specifically, we evaluate the hypothesis that the body-enclosing 'faecal case’ created and lived in by the larvae of *Neochlamisus* leaf beetles reduces their risk of predation. We especially focus on the complex case of *N. platani*, which is externally covered with host plant trichomes, and that includes a distinct trichome-filled chamber (‘attic’) in the case apex. Here, we separately evaluate the effects of case, trichomes, and attic on each of several behavioural stages of attack using *N. platani* and *N. bimaculatus* larvae and pupae. Three generalist predators (crickets, soldier bugs, and lynx spiders) that represent different feeding strategies were used in our individual-level repeated observation behavioural trials. Results strongly demonstrated that the case itself greatly reduces predation risk for all combinations of beetle species, life history stage, and predator. Additional evidence indicated that both trichomes and attics can further and independently reduce risk. Variation in results among treatments was also informative. For example, case components proved impervious to the piercing and sucking predator and often delayed mortality under attack by chewing crickets.

Patterns of predator behaviour provided no evidence that caseless larvae have alternative means of defence. However, continuous observation assays illustrated larval behavioural strategies in which the case was used to discourage predators. We further discuss the implications of our findings for *Neochlamisus* biology and architecture-associated behavioural evolution.

Introduction

Animal architecture can be thought of as "the application of behaviour to materials" and often enables a builder to expand control over some aspect of its environment (Hansell 2000; 2005 p. 33). Animals of a variety of taxa exhibit amazing feats of architecture in a multitude of media. One bizarre type of animal architecture is the building of structures out of an animal’s own waste materials. Perhaps the most elaborate example of such faecal architecture belongs to casebearing beetles known as the Camptosomata (Coleoptera: Chrysomelidae). Most camptosomate cases are initially constructed by an ovipositing female that uses individual plates of faeces to build a sealed, bell-shaped structure around each egg immediately after it is laid (Erber 1988; Brown & Funk 2005). Larvae later cut away the distally flattened “roof” of this "egg case", thus creating an opening from which they extend their heads and legs for feeding and movement, while the rest of the body remains within the case. (LeSage 1984; Erber 1988; Brown & Funk 2005) (Fig.1). Camptosomate larvae never leave their cases, but systematically add their own faeces to enlarge them and thus accommodate growth (see details in Brown & Funk 2005). Prior to pupation, the case opening is sealed and the case

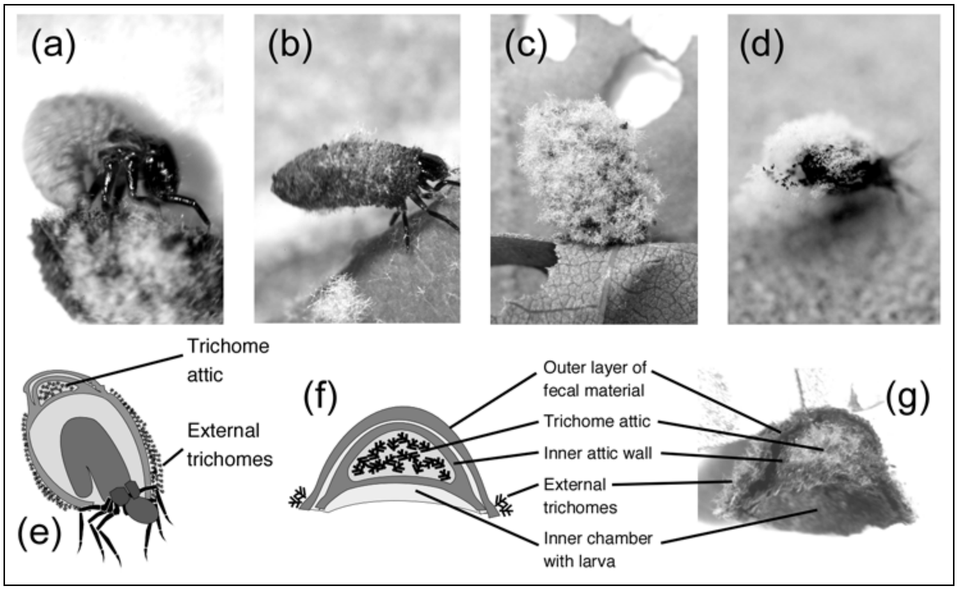

Figure 4-1. (a – d) Typical appearance of *N. platani* larvae: (a) without a case, (b) in a case with few external trichomes, (c) in a case with dense external trichomes, and (d) in a case from which the 'trichome attic' has been removed (i.e. note dark area in apex of case). (e) Diagrammatic representation of a larva in a case with a trichome attic. (f) Diagram of component parts of a trichome attic. (g) Cross section of a trichome attic from a pupal case. Parts of this figure have been modified from Figs. 4, 7 in Brown & Funk (2005).

attached to the substrate. During this immobile stage of the life cycle, pupae are particularly vulnerable to predation, and eviscerated pupal cases are observed in the field (Flinte and Macêdo 2004; DJF pers. obs.). Ultimately, the mature adult cuts itself free of its case with its mandibles.

One camptosomate, *Neochlamisus platani*, incorporates additional structural aspects into its case. Specifically, cases of *N. platani* are, to varying degrees, fuzzy in appearance due to an abundance of plant hairs (i.e. trichomes) that are incorporated into the faecal matrix of case walls and also attached externally (Popenoe & Marlatt 1899; Brown & Funk 2005; Chaboo et al. 2008) (Fig. 1c). Other species of *Neochlamisus* incorporate trichomes in their cases, but rarely to the degree of *N. platani* (Chaboo et al. 2008). These short, stellate, nonglandular trichomes derive from the leaves of *N. platani*'s host plant, the American sycamore, *Platanus occidentalis.* The trichomes of various plants function as a physical or chemical barrier that deters small arthropods from feeding on the plant surface (Bernays 1991; Valverde et al. 2001; Andres & Connor 2003), sometimes even killing these would-be herbivores (Gilbert 1971). However, some insects are able to bypass these defences (Moran 1986; Medeiros & Moreira 2002). *N. platani*, for example, are not deterred by the plant hairs of sycamore, instead clearing paths through them with their mandibles in order to feed (Brown and Funk in prep.). *Neochlamisus platani* performs another building behaviour that has not otherwise been documented in casebearers and that is known to be absent in other *Neochlamisus* (Brown & Funk 2005; Chaboo et al. 2008). Specifically, a cross section of the apex of pupal cases reveals an extra internal compartment, or "attic," that is filled with trichomes (Brown & Funk 2005) (Fig. 1e-g). This compartment sits above the main chamber that houses the pupa, from which it is separated by a thin layer of faecal material. The method by which this attic is constructed is not known.

These faecal cases are unique, even among insects. Faeces are typically discarded by insects since they can stimulate growth of harmful fungi and bacteria and attract predators and parasites (Müller and Hilker 1999; Weiss 2003, 2006). However, faeces can be advantageously used in diverse ways (e.g. Seymour 1974; Weaver et al. 1989; Anbutsu & Togashi 2002; Grasso et al. 2005; Borg-Karlson et al. 2006). As a building material, faeces share advantages with other secreted substances, requiring no production or collecting costs, as all organisms must produce waste, and providing uniform and malleable building materials (Olmstead and Denno 1992; Hansell 2005). Additionally, *Neochlamisus* faeces include undigested trichomes (CGB pers. obs.). Such composite materials, as in reinforced concrete, may better resist breaking under tension and compression (Hansell 2005). Faeces may also exhibit the same thixotropic properties as mud, a material used by many animal builders.

None the less, building, maintaining, and carrying a case throughout larval development, as in *Neochlamisus*, is itself likely time consuming and energetically costly (e.g. Stevens et al. 1999; Venner et al. 2003; McKie 2004; Hansell 2005). In addition, females are vulnerable during the lengthy (≥ half-hour, Brown and Funk 2005) period required for the construction of each of the dozens of individual egg cases that they construct (Funk 1998). The existence of the casebearing habit despite such costs hint at the likely existence of compensating benefits, yet we know of only three (modest-scale) prior studies that have investigated any possible beneficial functions for camptosomate faecal cases (Wallace 1970; Root & Messina 1983; Flinte & Macêdo 2004). Thus, the potential fitness advantages underlying the evolutionary origins and maintenance of this complex behaviour remain almost entirely unexplored. Animal constructions often aid in protection (from both biotic and abiotic factors), prey capture, and communication (Hansell 2005). For example, animal architectures can protect builders or their young from attack by reducing detection, as in birds that add lichen to their nest to disrupt its outline (Hansell 1996), or by reducing the probability of capture after detection, as in the funneled burrow entrances of *Paralastor* wasps (Smith 1978). Notably, several lineages of non-casebearing leaf beetles (Chrysomelidae) are known to retain faecal material as protective, if less complex, coverings for their eggs and larvae (Olmstead 1994; Müller & Hilker 2003; 2004; Chaboo et al. 2008). "Faecal shields", for example, present physically (Eisner et al. 1967; Olmstead and Denno 1993; Eisner & Eisner 2000; Nogueira-de-Sá & Trigo 2002) or chemically repellent barriers (Morton & Vencl 1998; Vencl and Morton 1998; Müller & Hilker 1999; 2003) to predators.

The faecal cases of casebearers such as *Neochlamisus*, as well as the external trichomes and trichome attics of *N. platani*, might similarly protect their builders from arthropod predators, reducing access to the otherwise ill-protected immatures, whose soft bodies have fewer hairs, spines, and hard sclerotized plates than other juvenile leaf beetles (Moldenke 1971; LeSage 1982; Root & Messina 1983). Since trichomes protect plants from herbivorous arthropods, incorporating them into faecal cases may similarly protect *N. platani* from predaceous arthropods. The trichome attic might further increase physical protection by presenting predators with a concentration of these trichomes and by increasing the faecal barriers that must be penetrated to gain access through the case apex. Field-collected pupal cases of various *Neochlamisus* taxa show damage to this particular region of the case, suggesting that it is indeed a site where predators seek entry (DJF pers. obs).

Camptosomate faecal cases have long intrigued biologists and a protective function is often assumed (e.g. Riley 1874; Cockerell 1891, Marlott & Propenoe 1899; Donisthorpe 1902). Here we provide the first in a series of studies that aim to elucidate what, if any, functional and potentially adaptive benefits *Neochlamisus* casebearers may gain from their unusual building behaviours. Most specifically, we test the long-standing hypothesis that these cases reduce the success of predator attack. Furthermore, we separately evaluate the contributions of faecal cases per se, trichome incorporation, and the trichome attic to antipredator defence. Using manipulative repeated-observation experiments, we quantify the threat posed by three disparate arthropod predators to active larvae and immobile pupae of *Neochlamisus*. We also report on specific predator and prey behaviours adopted during their confrontations in continuous-observation assays. We discuss our findings in the context of their implications for *Neochlamisus* biology in nature and for the evolution of these remarkable examples of animal architecture.

Methods

Study Animals

Neochlamisus platani and *N. bimaculatus* are ca. 4mm, univoltine leaf beetles, each of which develop and live entirely on one host plant, American sycamore and blackberry (*Rubus* spp.), respectively, across the American southeast (Karren 1972). Eggs, larvae, and adults were collected from host plants in and around Davidson

County, TN, during the springs of 2006 and 2007. These animals were placed on host foliage in plastic boxes lined with moistened paper towels in an incubator at 25 C and a 14:10 light:dark schedule at Vanderbilt University, Nashville, TN. Ovipositing adults and developing immatures were maintained in these containers to provide material for our experiments. Tests involved two life stages that are similar in size, but differ in mobility and case completion: last-instar larvae, whose head and legs can emerge from an opening in the case that enables feeding and walking, and immobile pupating animals in cases cemented to the substrate such that their openings are completely sealed (Fig. 1). *N. platani* and *N. bimaculatus* cases differ primarily in that the former have external trichomes and the trichome attic, while the latter does not. These closely related species commonly live together in the same habitats and thus may experience similar selective pressures and predators.

Since little is known about the natural predators of casebearers (Karen 1964; Cox 1996), three generalist predators of insects, representing the variety of predatory taxa and feeding morphologies present in the beetles' habitats, were chosen for our experiments: (1) The common house cricket, *Acheta domestica,* is a large biting/chewing insect with robust mandibles. These were purchased and subsequently maintained on bread and potatoes. (2) The spined soldier bug, *Podisus maculiventris,* is a native hemipteran that uses its beaklike piercing/sucking mouthparts to penetrate the cuticle of prey and consume their fluids. These were purchased as nymphs and reared to maturity on mealworms. (3) Green lynx spiders, *Peucetia* sp., are sit-and-wait predators with chelicerae that inject digestive fluids into prey, then consume it. These were collected from *Rubus* plants at one of the sites where *N. bimaculatus* were also collected for our study. Spiders were maintained individually on mealworms. The availability of *N. platani* larvae and pupae and of *N. bimaculatus* pupae was low when spiders were collected, preventing our evaluation of these predator-prey combinations.

Experimental Treatments

Except as noted above, individuals of each predator were paired with an individual *N. bimaculatus* larva or pupa representing one of two experimental treatments: (1) larva/pupa removed from its faecal case (i.e. 'caseless'), or (2) larva/pupa in its unmanipulated case (which, again, naturally possesses no external trichomes or attic). By contrast, *N. platani*'s more complex case architecture encouraged the evaluation of additional treatments: (1) caseless, (2) both trichomes and attic removed (3) trichomes removed but attic intact, (4) trichomes intact but attic removed, and (5) unmanipulated case with trichomes and attic intact (Figs. 1-2).

All test animals were removed from their rearing containers and haphazardly divided into treatments on the day their particular trials were conducted. For caseless treatments, cases were cut away from living larvae and pupae with forceps and a razor blade. These animals are not physically attached to their cases and were not harmed during case removal. For trichome-free treatments, the external layer of trichomes was gently removed with a scalpel (Fig. 1b). For attic-free treatments, the outer wall of the attic and the trichomes inside were removed with a scalpel and forceps, respectively, while the inner faecal layer separating the attic from the encased animal was left intact (Fig. 1d). Unmanipulated cases (Fig. 1c) were rotated between the fingers for several seconds to simulate and control for the physical manipulation of other treatments. All individuals were handled while wearing latex gloves to avoid exposure to human-derived substances.

Experimental Design and Protocols

All trials took place in a walk-in environmental chamber at 24 C and greater than 80% relative humidity. Each trial arena consisted of two 10 cm Petri dish bottoms taped together to form an enclosed chamber with adequate room for larvae and predators to move naturally. This had a floor of paper towel cut to fit the dish, to which 300 µl of water was added to prevent desiccation. All predators were starved for more than 48 h prior to use in a trial. Each predator was acclimated to its arena for two hours prior to testing. Each cricket was used in only a single trial. Soldier bugs were used in less than three trials. To avoid any systematic effects of previous experience, bug individuals used in multiple trials were distributed haphazardly across prey species and treatments and rested an average of 12 days between trials. Most spiders were used in one trial but a few were used in two, with 24 days between trials. No larva or pupa was used in more than one trial. A maximum of 30 arenas was observed at a time.

Repeated observation trials

To begin each trial period, one larva/pupa of a given experimental treatment was placed in the center of the arena with its resident predator (Fig. 2). Observations were then recorded every five minutes for two hours. During each observation, the level of threat posed to the larva/pupa by the predator was scored on a scale of 1-5 as follows: 1 = predator ignoring larva/pupa; 2 = predator approaching larva/pupa; 3 = predator ‘investigating’ larva/pupa (i.e. touching it with legs, antennae, or mouthparts); 4 = predator attacking larva/pupa (i.e. biting or piercing it); and 5 = predator killed larva/pupa. When an animal was killed prior to the conclusion of the trial, a value of 5

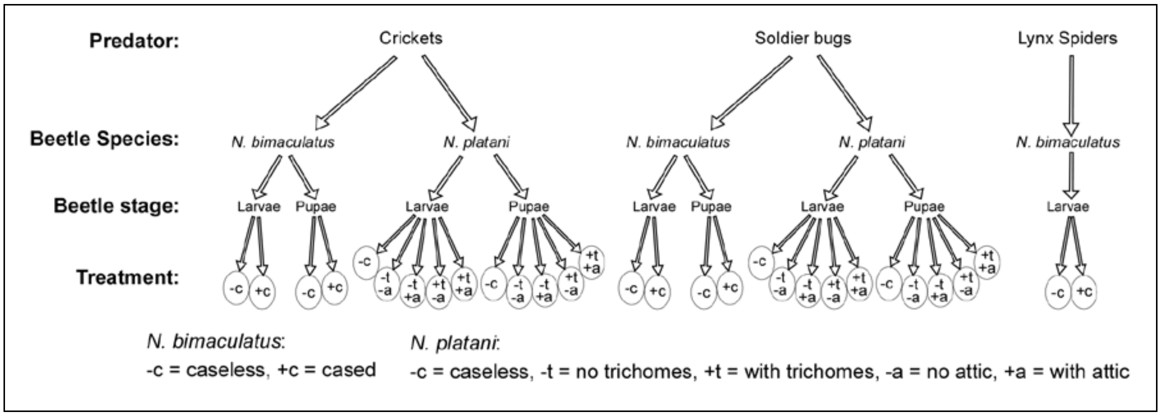

Figure 4-2. Basic structure of the experimental design for the repeated observation trials. Treatments are defined in the key at the bottom of the figure. See text for further details.

was entered for the remaining observations, thus accounting for the rapidity with which death occurred in evaluating threat level (see below). Sample sizes are provided in tables.

Attic/no-attic repeated observation choice trials

In the hope of more powerfully teasing out any effects of the trichome attic in reducing predation threat, choice tests were conducted using crickets and pairs of pupal *N. platani* ($N = 42$ pairs). White craft glue was used to attach two cased pupae in their natural orientation, near the center of a filter paper placed on the bottom of the arena. For one pupa in each pair, the trichome attic was intact and for the other it was removed. External trichomes were removed from both pupal cases to better isolate attic effects. Threat levels to each pupa from a cricket in the arena were scored every five minutes for two hours.

Continuous observation trials

Since no one has closely observed the behavioural interactions of any casebearer with its predators, we performed continuous observations of *N. platani* with crickets. Three replicate larvae from each of the treatments were individually exposed to crickets as above. Each larva was continuously observed for one hour or until killed. We continuously observed and recorded the behaviours of the predator and the larva, focusing on potentially defensive larval behaviours and predator responses. The timing of both predator and larval behaviours were recorded with a digital timer. The cricket in one of the caseless treatments died during the trial, which was thus excluded from evaluation.

Statistical analyses

Several kinds of information were extracted and analysed from the data collected in our repeated-observation experiments. First, the 24 threat levels recorded across

Table 4-1**.** Percentages of immatures that experienced varying levels of threat (investigation, attack, death) as a function of beetle species, case-associated trait, predator, and life history stage ("pooled" analyses combined data from larvae and pupae).

Predator/ Beetle Stage	n		% Investigated				% Attacked				% Killed			
	-	+	-	+	*G*	*P*	-	+	*G*	*P*	-	+	*G*	*P*
N. bimaculatus														
CASE														
Crickets														
Larvae	10	10	100	70	4.70	**0.03**	100	40	10.97	**0.0009**	100	10	21.02	**<0.0001**
Pupae	10	10	100	90	1.44	0.23	100	80	3.00	*0.08*	100	80	3.00	*0.08*
Pooled	20	20	100	80	5.99	*0.07*	100	60	13.11	**0.0002**	100	45	19.53	**<0.0001**
Soldier Bugs														
Larvae	4	4	100	75	1.53	0.22	100	50	3.45	*0.06*	100	0	11.09	**0.0009**
Pupae	4	4	75	50	0.54	0.46	75	0	6.09	**0.01**	50	0	3.45	*0.06*
Pooled	8	8	88	62	1.38	0.12	88	25	6.90	**0.004**	75	0	12.17	**0.0003**
Lynx Spiders														
Larvae	12	11	83	82	0.01	0.92	75	0	17.29	**<0.0001**	75	0	17.29	**<0.0001**
N. platani														
CASE														
Crickets														
Larvae	17	64	100	88	4.00	**0.05**	94	55	11.02	**0.0009**	94	47	14.71	**0.0001**
Pupae	15	60	93	83	1.12	0.29	93	58	8.00	**0.005**	93	55	9.18	**0.002**
Pooled	32	124	97	86	4.00	**0.02**	94	57	18.89	**<0.0001**	94	51	23.62	**<0.0001**
Soldier Bugs														
Larvae	13	52	100	87	3.33	*0.07*	100	79	5.44	**0.02**	92	31	17.62	**<0.0001**
Pupae	4	16	75	25	3.40	*0.07*	75	13	5.94	**0.02**	50	0	7.46	**0.006**
Pooled	17	68	94	72	4.58	**0.02**	94	63	7.63	**0.003**	82	24	20.33	**<0.0001**
TRICHOMES														
Crickets														
Larvae	32	32	90	84	0.58	0.45	56	53	0.06	0.80	50	44	0.25	0.62
Pupae	30	30	83	83	0.00	1.00	70	47	3.40	*0.07*	70	40	5.54	**0.02**
Pooled	62	62	87	83	0.26	0.30	63	50	2.11	*0.07*	60	42	3.93	**0.02**
Soldier Bugs														
Larvae	26	26	88	85	0.17	0.68	77	81	0.12	0.27	27	35	0.36	0.45
Pupae	8	8	25	25	0.00	1.00	0	25	3.06	0.92	0	0	0.00	1.00
Pooled	34	34	74	71	0.07	0.39	59	68	0.57	0.77	21	27	0.33	0.72
ATTICS														
Crickets														
Larvae	32	32	78	97	5.71	0.98	50	59	0.58	0.55	50	44	0.25	0.61
Pupae	30	30	93	73	4.58	**0.03**	67	50	1.72	0.19	63	47	1.69	0.19
Pooled	62	62	86	86	0.00	1.00	58	55	0.13	0.36	57	45	1.58	*0.10*
Soldier Bugs														
Larvae	26	26	85	89	0.17	0.32	81	77	0.12	0.73	27	35	0.36	0.45
Pupae	8	8	38	13	1.38	0.24	13	13	0.00	1.00	0	0	0.00	1.00
Pooled	34	34	78	71	0.07	0.40	65	62	0.06	0.40	11	27	0.33	0.72

Differences in percentages in the absence (-) versus presence (+) of each trait were calculated using goodness of fit tests (df = 1). Significant differences are in bold and marginally non-significant differences ($0.10 \geq P > 0.05$) are italicized. For these data, effectiveness of a trait at reducing predation success is suggested by a lower percentage in the "+" column than in the "-" column.

Table 4-2. Mean threat level experienced by immatures as a function of beetle species, case-associated trait, predator, and life history stage (“pooled” analyses combined data from larvae and pupae).

Predator/	n		Mean Threat			
Beetle stage	-	+	-	+	*H*	*P*
N. bimaculatus						
CASE						
Crickets						
Larvae	10	10	4.7 + 0.10	1.4 + 0.19	14.45	**<0.0001**
Pupae	10	10	4.3 + 0.29	3.3 + 0.48	3.30	**0.03**
Pooled	20	20	4.5 + 0.16	2.4 + 0.34	17.67	**<0.0001**
Soldier Bugs						
Larvae	4	4	4.8 + 0.00	1.2 + 0.13	6.05	**0.007**
Pupae	4	4	2.9 + 0.96	1.2 + 0.11	1.40	0.12
Pooled	8	8	3.8 + 0.58	1.2 + 0.08	6.37	**0.006**
Lynx spiders						
Larvae	12	11	3.2 + 0.43	1.2 + 0.05	7.53	**0.003**
N. platani						
CASE						
Crickets						
Larvae	17	64	4.5 + 0.23	2.6 + 0.21	15.58	**<0.0001**
Pupae	15	60	4.0 + 0.33	3.0 + 0.23	2.61	*0.06*
Pooled	32	124	4.3 + 0.20	2.8 + 0.20	16.58	**<0.0001**
Soldier Bugs						
Larvae	13	52	4.3 + 0.26	2.3 + 0.17	15.14	**<0.0001**
Pupae	4	16	2.5 + 0.81	1.1 + 0.07	3.87	**0.02**
Pooled	17	68	3.8 + 0.32	2.0 + 0.14	15.72	**<0.0001**
TRICHOMES						
Crickets						
Larvae	32	32	2.8 + 0.31	2.4 + 0.28	1.32	0.12
Pupae	30	30	3.6 + 0.31	2.5 + 0.31	3.90	**0.02**
Pooled	62	62	3.2 + 0.22	2.5 + 0.21	5.37	**0.01**
Soldier Bugs						
Larvae	26	26	2.2 + 0.22	2.5 + 0.27	0.04	0.58
Pupae	8	8	1.0 + 0.01	1.2 + 0.13	0.06	0.60
Pooled	34	34	1.9 + 0.19	2.2 + 0.22	0.09	0.62
ATTICS						
Crickets						
Larvae	32	32	2.4 + 0.29	2.7 + 0.30	1.31	0.87
Pupae	30	30	3.4 + 0.32	2.7 + 0.33	3.53	**0.03**
Pooled	62	62	2.9 + 0.22	2.7 + 0.22	0.18	0.34
Soldier Bugs						
Larvae	26	26	2.3 + 0.25	2.4 + 0.24	0.004	0.53
Pupae	8	8	1.2 + 0.12	1.1 + 0.08	1.06	0.15
Pooled	34	34	2.0 + 0.21	2.1 + 0.21	0.01	0.54

Mean differences (+ s.e.) in the absence (-) versus presence (+) of each trait were calculated using Kruskal-Wallis nonparametric t-tests (*H*). For all comparisons: *df* = 1. Significant differences are in bold and marginally non-significant differences ($0.10 \geq P > 0.05$) are italicized. For these data, effectiveness of a trait at reducing predation success is suggested by a lower mean threat in the “+” column than in the “-“ column.

Table 4-3. Timing between stages of predation threat as a function of beetle species, case-associated trait, predator, and life history stage ("pooled" analyses combined data from larvae and pupae).

Predator/ Beetle stage	n		Time to investigation				Time between investigation and attack				Time between attack and death				Survival time				Number of attacks escaped			
	-	+	-	+	*H*	*P*	-	+	*H*	*P*	-	+	*H*	*P*	-	+	*H*	*P*	-	+	*H*	*P*
N. bimaculatus																						
CASE																						
Crickets																						
Larvae	10	10	12 + 2.9	48 + 16.4	2.81	**0.05**	0 + 0.0	65 + 18.4	11.76	**0.0003**	6 + 1.2	55 + 30.5	0.82	0.18	17 + 3.2	116 + 4.5	15.83	<**0.0001**	0 + 0.0	0.8 + 0.5	5.38	**0.01**
Pupae	10	10	25 + 8.9	22 + 11.0	0.02	0.57	0 + 0.0	38 + 15.2	8.81	**0.002**	2 + 0.8	10 + 3.5	5.77	**0.008**	27 + 9.2	64 + 15.1	5.29	**0.01**	0 + 0.0	0.1 + 0.1	1.25	0.13
Pooled	20	20	18 + 4.8	35 + 10.1	1.39	0.12	0 + 0.0	50 + 11.9	20.76	<**0.0001**	4 + 0.8	25 + 11.4	4.76	**0.01**	22 + 4.9	90 + 9.7	19.84	<**0.0001**	0 + 0.0	0.3 + 0.2	5.33	**0.01**
Soldier Bugs																						
Larvae	4	4	5 + 0.0	51 + 25.1	3.94	**0.02**	0 + 0.0	22 + 21.7	1.33	0.12	25 + 0.0	105 + 10.0	4.80	**0.01**	30 + 0.0	120 + 0.0	7.00	**0.004**	0 + 0.0	1 + 0.0	5.00	**0.01**
Pupae	4	4	38 + 27.6	94 + 21.5	2.19	*0.07*	33 + 33.3	52 + 37.5	0.35	0.28	25 + 11.5	NA	NA	NA	81 + 22.9	120 + 0.0	2.29	*0.07*	0.3 + 0.3	NA	NA	NA
Pooled	8	8	21 + 14.2	73 + 17.3	5.21	**0.01**	14 + 14.3	34 + 18.4	1.58	*0.10*	25 + 4.4	105 + 10.0	5.04	**0.01**	56 + 14.4	120 + 0.0	8.73	**0.002**	0.1 + 0.1	1 + 0.0	4.57	**0.02**
Lynx spiders																						
Larvae	12	11	47 + 12.0	53 + 13.5	0.10	0.38	9 + 8.5	82 + 11.2	11.86	**0.0003**	25 + 0.0	NA	NA	NA	73 + 10.6	120 + 0.0	11.98	**0.0003**	0 + 0.0	NA	NA	NA
N. platani																						
CASE																						
Crickets																						
Larvae	17	64	12 + 2.5	34 + 4.9	3.10	**0.03**	6 + 4.8	39 + 6.4	7.95	**0.003**	6 + 1.6	20 + 6.3	1.20	0.14	24 + 6.6	79 + 6.0	18.08	<**0.0001**	0.06 + 0.1	0.15 + 0.1	0.72	0.20
Pupae	15	60	24 + 7.5	36 + 5.2	0.32	0.29	8 + 7.5	28 + 6.0	4.51	**0.02**	7 + 4.9	10 + 2.7	11.06	**0.0005**	37 + 10.0	65 + 6.5	3.68	**0.03**	0.07 + 0.1	0.14 + 0.1	0.47	0.25
Pooled	32	124	17 + 3.8	35 + 3.6	3.67	**0.03**	7 + 4.2	34 + 4.4	12.12	**0.0003**	7 + 2.4	15 + 3.4	9.02	**0.001**	30 + 5.9	72 + 4.5	19.51	<**0.0001**	0.07 + 0.1	0.14 + 0.0	1.19	0.14
Soldier Bugs																						
Larvae	13	52	13 + 1.9	34 + 5.5	1.73	0.10	0 + 0.0	16 + 4.4	5.58	*0.09*	32 + 6.5	70 + 5.8	9.00	**0.001**	45 + 6.6	103 + 4.4	20.39	<**0.0001**	0.08 + 0.1	1.00 + 0.2	10.61	**0.0005**
Pupae	4	16	68 + 26.7	101 + 9.9	2.78	**0.05**	0 + 0.0	44 + 20.1	3.08	**0.04**	32 + 12.0	68 + 42.5	0.79	0.19	91 + 20.0	120 + 0.0	8.42	**0.002**	0.00 + 0.0	0.50 + 0.5	1.50	0.11
Pooled	17	68	26 + 8.2	50 + 5.9	2.40	*0.06*	0 + 0.0	18 + 4.4	7.73	**0.003**	32 + 5.6	69 + 5.7	10.70	**0.0005**	56 + 8.2	107 + 3.5	24.45	<**0.0001**	0.06 + 0.1	0.98 + 0.2	13.07	**0.0002**
TRICHOMES																						
Crickets																						
Larvae	32	32	35 + 6.7	32 + 7.3	0.70	0.80	34 + 8.3	45 + 9.9	0.54	0.23	12 + 6.5	28 + 10.6	0.24	0.31	73 + 9.1	85 + 7.8	0.81	0.18	0.06 + 0.1	0.24 + 0.1	2.05	*0.08*
Pupae	30	30	30 + 7.1	41 + 7.7	1.53	0.11	17 + 7.0	40 + 9.4	2.72	**0.05**	6 + 0.8	15 + 6.4	2.00	*0.08*	49 + 8.7	82 + 8.9	6.52	**0.005**	0.14 + 0.1	0.14 + 0.1	0.00	1.00
Pooled	62	62	33 + 4.8	37 + 5.3	0.04	0.42	26 + 5.5	43 + 6.8	2.81	**0.05**	9 + 2.9	22 + 6.5	1.79	*0.09*	61 + 6.4	83 + 5.9	6.21	**0.006**	0.11 + 0.1	0.19 + 0.1	1.06	0.15
Soldier Bugs																						
Larvae	26	26	33 + 7.6	35 + 8.1	0.001	0.49	19 + 7.0	13 + 5.3	0.14	0.65	72 + 8.7	67 + 7.9	0.33	0.72	106 + 5.7	100 + 6.7	0.88	0.82	1.05 + 0.3	0.95 + 0.2	0.13	0.64
Pupae	8	8	109 +	93 +	0.08	0.61	45 +	43 +	0.00	1.00	NA	68 +	NA	NA	120 +	120 +	0.00	1.00	NA	0.50 +	NA	NA

			8.8	18.0			25.0	2.5				42.5			0.0	0.0				0.5		
Pooled	34	34	51 + 8.2	49 + 8.5	0.12	0.63	21 + 6.7	15 + 5.7	0.33	0.72	72 + 8.7	67 + 7.7	0.29	0.71	109 + 4.5	104 + 5.3	0.80	0.81	1.05 + 0.3	0.91 + 0.2	0.23	0..68
ATTICS																						
Crickets																						
Larvae	32	32	43 + 7.9	25 + 5.4	4.15	0.98	45 + 10.4	35 + 8.1	0.17	0.66	5 + 0.5	33 + 11.0	4.74	**0.01**	80 + 8.3	78 + 8.8	0.05	0.59	0.00 + 0.0	0.28 + 0.1	5.06	**0.01**
Pupae	30	30	28 + 6.1	43 + 8.4	1.97	0.80	25 + 7.5	32 + 9.8	0.60	0.22	8 + 2.0	11 + 5.7	0.20	0.33	57 + 9.1	74 + 9.3	1.86	*0.09*	0.15 + 0.1	0.13 + 0.1	0.02	0.55
Pooled	62	62	36 + 5.1	34 + 5.1	0.29	0.71	34 + 6.4	34 + 6.2	0.14	0.36	7 + 1.2	23 + 6.7	1.95	*0.08*	69 + 6.3	76 + 6.4	0.56	0.23	0.08 + 0.1	0.21 + 0.1	2.27	*0.07*
Soldier Bugs																						
Larvae	26	26	31 + 8.0	38 + 7.6	1.73	*0.09*	12 + 5.9	20 + 6.5	0.28	0.30	78 + 8.3	61 + 7.8	2.85	0.95	104 + 6.1	101 + 6.4	0.10	0.63	1.00 + 0.3	1.00 + 0.2	0.08	0.39
Pupae	8	8	95 + 15	106 + 14.9	0.94	0.17	30 + 20.8	85 + NA	1.80	*0.09*	110 + NA	25 + NA	1.00	0.84	120 + 0.0	120 + 0.0	0.00	1.00	1.00 + NA	0.00 + NA	1.00	0.16
Pooled	34	34	46 + 8.4	54 + 8.3	1.14	0.14	14 + 5.7	22 + 6.8	0.26	0.31	80 + 8.1	59 + 7.6	3.93	0.98	108 + 4.8	106 + 5.1	0.09	0.62	1.00 + 0.3	0.95 + 0.2	0.01	0.53

Mean differences (+ s.e.) in the absence (-) versus presence (+) of each trait were calculated using Kruskal-Wallis nonparametric t-tests (*H*). For all comparisons: *df* = 1. Significant differences are in bold and marginally non-significant differences ($0.10 \geq P > 0.05$) are italicized. For these data, effectiveness of a trait at reducing predation success is suggested by a higher value in the “+” column than in the “-“ column.

observation intervals during a trial for each individual larva/pupa were averaged to obtain a trial-level “mean threat” value. Second, we separately quantified the proportion of test animals that were investigated, attacked, and killed during the trials. Third, we determined the mean amount of time between which successive threat levels were reached. The time between trial initiation and initial investigation by the predator was interpreted as reflecting ‘long-distance' attraction to, or recognition of, the larva/pupa by the predator. Likewise, the time between initial investigation and attack was interpreted in terms of the same factors following contact. Last, the time to death after initial attack was evaluated as the speed or ‘handling time’ required by a predator to dispatch its prey. Time to death and number of attacks survived provided additional data on the success of prey resistance.

All analyses used nonparametric methods due to deviations of the data from normality. We used Kruskal-Wallis test to evaluate differences among treatments in continuous variables (Dunn 1964; Zar 1996). Proportions were analysed using G-tests to evaluate goodness of fit. For the attic/no attic choice test, we performed a Wilcoxon's signed-ranks test for two groups, with the data arranged as paired observations (Sokal & Rohlf 1995). Since we a priori hypothesized that each faecal case component would independently lower predation risk, all tests with two treatments were one-tailed. Means are presented + standard errors. Statistical tests were not conducted for the continuous observation trials, which provided qualitative insights on behaviours relevant to this predator-prey interaction. “Tendencies” refer to tallied results from means comparisons across our analyses, and were inspected to evaluate general patterns (e.g. “in X of Y analyses, cases with trichomes were less affected by predation than those without, illustrating a tendency”). We have not statistically analysed these tendencies due to issues of non-independence. Any finding described below that does not refer to a tendency represents a statistically significant result. The exception to this are the results from the continuous observation trials.

Results

Repeated Observation Trials

General patterns

All three predators readily ate both species and life stages of *Neochlamisus* without cases, indicating their general acceptability as prey and lack of effective case-independent defence mechanisms. With respect to cased individuals, crickets proved capable of chewing through cases to remove larvae/pupae and consume them. By contrast, soldier bug beaks appeared unable to pierce case walls. While bugs sometimes succeeded at inserting their beaks into larval case openings to reach their prey, larvae often escaped injury by holding the case down flush to the substrate. No spider was observed to attack or kill a cased individual (Table 1). For all species, stages and predators, cased individuals experienced lower threat than caseless ones in all but one analysis (exception: pupae facing soldier bugs, $P = 0.12$), despite sometimes-modest sample sizes (Table 2). Further analyses of *N. platani* failed to detect significant threat level differences among the four cased treatments. (For these, 2-tailed tests were used given our lack of a priori hypotheses on the relative anti-predatory contributions of different aspects of case architecture.) Therefore, we pooled treatments sharing the same architectural aspect and made the following comparisons of the presence versus absence of individual factors: (1) cased versus caseless, (2) external trichomes present versus removed, and (3) attic present versus removed.

N. bimaculatus

Faecal cases

Cased larvae were less likely to be investigated by crickets than caseless larvae, but cases did not have this effect for pupae or against other predators (Table 1). In the majority of analyses, cases reduced the likelihood of attack and death (Table 1), lowered mean threat (Table 2), and increased survival time (Table 3). In fact, soldier bugs and spiders never killed *N. bimaculatus* in cases (Table 1). Crickets and soldier bugs took longer to investigate cased larvae (Table 3). Crickets attacked caseless larvae and pupae almost immediately after investigation, but took much longer to attack cased larvae/pupae and longer to kill cased pupae (Table 3). Cases also increased handling time and total survival time of larvae against soldier bugs (Table 3). Tendencies show that faecal cases of *N. bimaculatus* lowered predation success in all but one of the analysed comparisons (40/41) (Table 4).

N. platani

Faecal cases

Cases lowered the proportion of *N. platani* larvae that were investigated by crickets and by crickets and soldier bugs when larvae and pupae are pooled (Table 1). Cases greatly lowered the likelihood of being attacked and killed by crickets and soldier bugs in all comparisons (Table 1). Cased larvae/pupae experienced lower threat from both predators, although results were marginally nonsignificant when pupae faced soldier bugs (Table 2). Crickets took longer to investigate cased larvae while soldier bugs took longer to investigate cased pupae (Table 3). Both predators took longer to attack after investigating cased larvae/pupae in all but one situation (Table 3). Cases also increased predator handling time for crickets attacking pupae and for soldier bugs attacking larvae (Table 3). Cases increased survival time in all comparisons and cased larvae escaped more attacks per trial when facing soldier bugs (Table 3). A tendency for *N. platani* to benefit from the presence of a case was detected in all comparisons (36/36; Table 4).

Trichomes

External trichomes benefited pupal but not larval *N. platani* when exposed to crickets (Tables 1-3). Trichomes lowered the proportion of pupae killed (Table 1) and mean threat (Table 2), and increased the time between investigation and attack as well as survival time (Table 3). Tendencies revealed a general increase in resistance of trichome-bearing animals to crickets (in 15/16 comparisons) but not soldier bugs (2/13 comparisons) (Table 4).

Trichome attics

Attics did not affect the proportion of larvae/pupae attacked or killed by either predator (Table 1), but did lower the proportion investigated (Table 1) and mean threat (Table 2) for pupae facing crickets. Larvae with attics also required longer handling times and escaped more often from crickets (Table 3). When facing soldier bugs, larvae with attics were more resistant in only 6/17 comparisons, while, contrarily, 12/15 pupal comparisons showed this tendency (Tables 4).

Table 4-4. Number of analyses (from Tables 1-3) in which a positive (Yes) versus negative (No) effect on resistance to predation was observed for each case-associated trait as function of predator, beetle species, and life history stage.

Beetle stage/ Treatment	Crickets		Soldier Bugs		Lynx Spiders		Sum	
	No	Yes	No	Yes	No	Yes	No	Yes
Larvae								
Cases								
N. bimaculatus	0	9	0	9	0	7	0	25
N. platani	0	9	0	9	-	-	0	18
Trichomes	1	8	7	2	-	-	8	10
Attics	6	3	5	3	-	-	11	6
Pupae								
Cases								
N. bimaculatus	1	8	0	7	-	-	1	15
N. platani	0	9	0	9	-	-	0	18
Trichomes	0	7	4	0	-	-	4	7
Attics	1	8	2	4	-	-	3	12
Sum of above:								
Cases	1	35	0	34	0	7	1	76
Trichomes	1	15	11	2	-	-	12	17
Attics	7	11	7	7	-	-	14	18

Results from each analysis were scored as “yes” if the mean antipredatory effect was positive and as “no” if it was negative, irrespective of statistical significance. Comparisons resulting in no difference (i.e. ties) are not included. These tallies illustrate tendencies that might not be apparent from individual statistical analyses based on modest sample sizes.

Attic/No-Attic Repeated Observation Choice Trials

Crickets did not investigate either of the paired cases in 9 of 42 trials. For the remaining 33 trials, pupae with attics suffered significantly lower threat from crickets than those lacking them (mean difference in threat = 0.09 + 0.15, Wilcoxon's signed-ranks test for pairs: $T = 226$, $N = 33$, $P < 0.005$) and were less frequently the first of the two pupae to be investigated (Goodness of fit test: 23/33 trials, $G = 5.3$, df =1, $P = 0.02$), attacked (18/27 trials, $G = 3.1$, df =1, $P = 0.04$), and killed (15/21 trials, $G = 4.0$, df =1, $P = 0.02$).

Continuous Observation Trials

Cased larvae were often quite active during these trials and several spent considerable time walking (Table 5), especially when not under attack (Fig. 3). These larvae also spent considerable time with their cases held down flush with the substrate, such that the case opening was inaccessible. Larvae in this position were observed to survive many bouts of investigation and even attack (Fig. 3) and experienced reduced predation threat 75% of the time (Table 5). This was the most common defensive behaviour of cased larvae. We had hypothesized that larvae might orient the case apex, which contains the fortified trichome attic, towards oncoming predators. A related behaviour is common in tortoise beetles, which wave their faecal shields at attackers (Eisner et al. 1967), and was suggested to be a defensive behaviour in *N. platani* by Neal (1989). However, we did not observe any such consistent orientation towards attackers. We did, however, observe two behaviours not previously described. First, two larvae shook the case back and forth (i.e. "case wiggling"), and one individual did this six times. This behaviour lowered attack threat within 30 s in 80% of occurrences (Table 5). It was often performed after a cricket began chewing on the case, perhaps startling or irritating it, thus providing short term

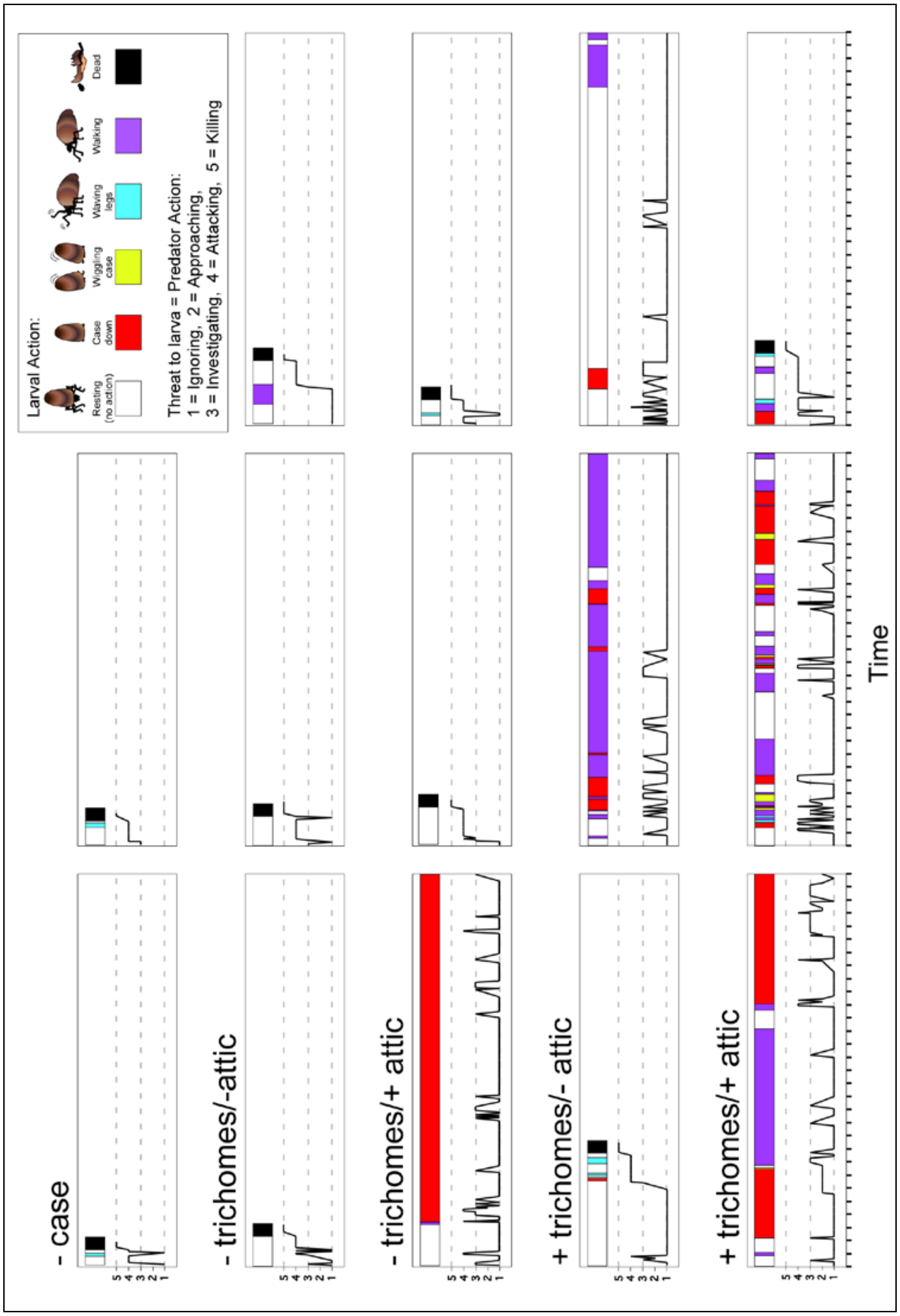

Figure 4-3. Ethograms of *N. platani* larval behaviour and cricket predatory activity from the 1-hr continuous observation trials. Larval behaviours over time are colour-coded in the top portion of each plot. Predator actions/threat-levels are indicated on a 1-5 scale at the bottom of each plot.

Table 4-5. Summary of behaviours performed in the continuous observation assay

	Resting	Case Down	Wiggling case	Waving legs	Walking
# of individuals performing behaviour	14/14	7/12	2/12	6/14	7/14
# of times behaviour was performed	36	21	7	10	34
# of times initiated in presence of predator	14	16	5	8	8
# of times behaviour reduced threat within 30s	1	12	4	2	4
Mean threat when behaviour was initiated	2.0 + 0.22	2.8 + 0.25	2.4 + 0.43	3.4 + 0.40	1.56 + 0.19
Mean threat 30s after behaviour was initiated	2.4 + 0.27	1.7 + 0.25	1.14 + 0.14	2.8 + 0.49	1.59 + 0.18

Mean threats are provided to show what threat level might have induced a given behaviour and what threat level resulted shortly after the behaviour was initiated.

relief from attack (Fig. 3). Second, both cased and caseless larvae were seen to lift their forelegs and wave them at an investigating cricket (Fig. 3). This behaviour reduced threat within 30 s in a minority of instances (Table 5) yet was repeatedly exhibited by multiple individuals, and at times of highest average threat compared to other behaviours.

Discussion

This study demonstrates the success of a complex and unique example of animal architecture – the faecal case of camptosomate leaf beetles – at resisting predation. It does so for each of two tested *Neochlamisus* species and both larval and pupal stages against three trophically disparate arthropod predators. While evidence for the antipredatory properties of the case itself proved highly compelling, we further demonstrate the additional contributions of external trichomes and the 'trichome attic' in the particularly intricate faecal case of *N. platani*. This study presents a rare quantitative experimental study of the presumed anti-predator properties of animal-built structures (Hansell 2005). More specifically, it represents the first detailed demonstration of a potentially adaptive function for these intriguing constructions. Besides being unusual in its choice of building medium, our study system presents an uncommon example of large-scale contributions of immatures to the creation of an animal architecture. It further offers a very rare and intriguing example whereby physical (rather than chemical) anti-herbivore plant structures have been co-opted for the herbivore's own defence. Here, we interpret the contributions of particular faecal case elements and larval behaviours to predator resistance and speculate on the fitness effects and evolution of this remarkable behavioural/architectural trait.

Faecal Cases

Primary defences lower rates of detection and attack by predators (Ruxton et al. 2004). *Neochlamisus* faecal cases appeared to provide a primary defence against crickets and soldier bugs, which often investigated cased larvae/pupae less frequently and quickly than caseless individuals. Here, this primary defence probably represents a visual and olfactory masquerade since cases resemble common, yet inedible components of the environment (Ruxton et al. 2004). Although some predators use faeces to locate prey (Müller & Hilker 2004; Weiss 2006), it seems unlikely that they would expect prey to be within the faeces itself. Indeed, even after investigating prey, predators in our study often took much longer ‘deciding’ to attack those with cases, thus increasing both predators’ handling times and opportunities for prey escape. In nature, faecal cases may play a yet larger role in primary defence than indicated by our studies since our small Petri dish test arena artificially increased the relative frequency of physical encounters between predators and cased prey. Indeed, it is also possible that faecal cases evolved to masquerade as buds or other host-plant-associated structures (Briggs 1905; DJF pers. obs). In addition, faecal cases may provide a cryptic covering for the bright-coloured larvae and pupae, which would otherwise visually contrast greatly with their host plants. These functions would best be tested against natural backgrounds.

Again bearing in mind arena space constraints, we note that the great majority of test larvae/pupae were none the less eventually investigated by predators, offering the opportunity to evaluate the potential role of faecal cases as secondary defences, that is, those that reduce attack success after detection (Ruxton et al. 2004). And, indeed, cases of both species greatly decreased the likelihood of attack and mortality, while also increasing predator handling time, in a large fraction of experiments. Cases thus clearly present a successful barrier to predators, while also reducing the energetic benefit to predator attack and offering increased opportunities for larval escape.

Although not investigated here, the secondary defence provided by *Neochlamisus* faecal cases could have a chemical component. Wet faecal coverings in some other leaf beetles protect larvae from ants via chemicals derived from the host plant rather than the faeces itself (Morton & Vencl 1998; Vencl & Morton 1998; Vencl et al. 1999). Storing such substances in externally maintained faeces reduces the need for anatomical and physiological mechanisms of sequestration (Hilker 1992). Additionally, many conventionally chemically sequestering species must be wounded before their defensive compounds are secreted (Olmstead 1994). By contrast, as faecal cases can easily be repaired (Brown & Funk 2005), a faecal means of storing chemicals would allow an individual's case (rather than the individual itself) to be sampled by a predator without permanent harm. Finally, caseless larvae were readily eaten by all predators, consistent with (though not demonstrating) a lack of case-independent chemical defence. However, the chemistry of camptosomate faecal cases and larvae is presently unknown and in need of investigation.

External Trichomes

The external trichomes on *N. platani* cases further increased defence against the biting/chewing crickets, predominately in pupae. Many plants use trichomes to protect their leaf surfaces from herbivory by irritating and/or limiting movement of small herbivores (Levin 1973). Some herbivores that can survive on pubescent plants indirectly benefit from trichomes that also inhibit their arthropod predators (Roda et al. 2000; Mulatu et al. 2004; Guershon & Gerling 2006). The case-associated trichomes studied here provided similar, but more direct, protection. Crickets were less likely to attack and kill larvae/pupae with trichome-covered cases, whether because the trichomes limited predator recognition of a food source, were irritating, or presented a physical barrier. Interestingly, trichomes were much less effective against soldier bugs, whose beaks lack the palps (mouthparts) that often bear chemo- and mechanoreceptors in other insects (Chapman 1998). Thus, perhaps the bugs suffer less trichome-induced irritation. Trichomes did not increase time to investigation by either crickets or soldier bugs. We suspect, however, that trichomes might indeed reduce case detection in the natural environment of a trichome-covered sycamore leaf, where mimicry of plant structures or camouflage may come into play.

Although co-opting (sequestering) chemicals from host plants as a source of defence may be common in insects (Eisner 2005), our study has identified an extremely rare example of an herbivore that co-opts physical attributes of host defence for its own protection. We could find very few examples to fit these criteria. These include an herbivorous amphipod that builds a protective shelter from its toxic algal host (Hay and Duffy 1990) and bagworms that use whole leaf clippings (rather than just the physical defences of the host) in making their bags (Rivers et al. 2002). More generally, although various animal taxa protect themselves by attaching parts of other organisms to their bodies (Millott 1955; Brooks 1988; Stachowicz & Hay 1999; Thanh et al. 2003; Boyero and Bernard 2004), considerably fewer actually collect parts of their food source for this purpose (e.g. Eisner et al. 1978; Stachowicz and Hay 2000; Brandt & Mahsberg 2002; Frick 2003). Amazingly, a juvenile lacewing also gathers and attaches sycamore trichomes, for protection from bugs with beaks too short to penetrate the trichome covering (Eisner et al. 2002). In this example, however, the insect is a predator rather than an herbivore, so its trichomes do not derive from a co-opted food source. Thus, beetle and lacewing may play opposing roles in the coevolutionary dynamics of sycamores and their insect associates.

Trichome Attic

One of our most intriguing findings was that the enigmatic, species-specific, trichome-filled chamber in the case apex of *N. platani* also contributed importantly to predation resistance. Interestingly, however, larvae did not appear to receive the attic-associated protection that pupae did. Although this has not been formally investigated, it seems likely that attics are not formed until the case is prepared for pupation during the last larval instar (the instar evaluated here). Otherwise this complex structure would require repeated rebuilding as the case was regularly enlarged to accommodate continual larval growth (Brown and Funk 2005). If so, attics may specifically represent an adaptation to protect the immobile pupal stage. That said, the mature larvae in our studies did sometimes benefit from their attics in a manner that pupae could not. Specifically, crickets were sometimes observed chewing on a removed attic while the larva escaped its attacker. In this way, the attic was reminiscent of a lizard's tail that can be autonomously shed to divert attention while the lizard escapes.

Defensive Behaviours Associated with Cases

As described in the Results, this study provides the first detailed observations of defensive behaviours of a larval casebearer, including previously undescribed strategies. As pupae are enclosed in immobile cases, they are not capable of such behaviours. However, the sealed openings and trichome attics of pupal cases may help compensate for this. Indeed, some larval behaviours appeared specifically aimed at preventing predator access to their vulnerable case openings. Many larvae stopped moving and held their cases down flush to the substrate in a 'case-down' position that has been previously observed to block predator attack in other camptosomates (e.g. Wallace 1970). Indeed, some predators (here, the bugs) cannot penetrate the case and thus can only access a larva via the case opening. The case-down behaviour may also promote masquerading. It was most often initiated when the predator was proximate, and seemed to reduce threat by diminishing predator interest. The occasional apical case wiggling of these individuals may function to dislodge or startle a predator and often yielded cricket retreat, and generally reduced threat level. The waving of forelimbs at cricket attackers may represent a behavioural preadaptation deriving from the evolution of strongly hooked tarsal claws to secure larvae to their host plants. Indeed, one larva was observed to 'scratch' a cricket's face, causing it to retreat. This behaviour appears to represent a last resort when under attack in high-threat situations.

Parasitoids

Neochlamisus can suffer high mortality due to parasitoid wasps (Brown 1943; Neal 1989; DJF unpubl. data), yet little is known about parasitoids of the Camptosomata.

Parasitoids are insects whose larvae develop on the living tissues of its host before eventually killing it. Parasitoids of casebearers are interesting, in part, because they must bypass the faecal case to deposit their eggs. This may occur either by penetrating the case with the egg-laying structure (ovipositor), a necessity for egg parasitoids (DJF pers. obs.), or by inserting the ovipositor into the case opening (Schöller 1999; DJF pers. obs.). Notably, the developing parasitoids of casebearers enjoy the same protective benefits of the faecal case as their host. Indeed, some *Neochlamisus* parasitoids wait until the pupal case has been sealed before completing development, providing added protection (DJF pers. obs).

Some parasitoids use faeces as kairomones to locate their prey (Schaffner & Müller 2001; Müller & Hilker 2003), so casebearers may be especially susceptible to such detection. Together, these observations support the intriguing idea that casebearers may contend with fitness trade-offs by building a structure that deters predators, but possibly increases parasitism. The evolutionary outcome of this tradeoff may depend in part on the capacity of parasitoids to adapt in response to the challenges presented by the case. Thus, the case might decrease parasitism by generalist parasitoids not morphologically or behaviourally capable of circumventing the case to oviposit on it or escape from it upon maturation. By contrast, specialist parasitoids may be more likely to adapt to these challenges by evolving sufficiently robust and lengthy ovipositors and associated behaviours (Danks 2002). Indeed, sampling across *Neochlamisus* species has so far revealed only two species of egg parasitoids (DJF, unpubl. data). Parasitoids as well as predators may additionally impose selection that yields tradeoffs involving case-wall thickness or the distance from the case wall to the immature within the case. While increasing these parameters may reduce access to the immature, this also increases the energetic costs of building and carrying a larger case. A classic example of a similar tradeoff involves parasitoid ovipositor length and the thickness of galls induced by the *Eurosta* fruit flies that develop within them (Weis et al. 1992). External trichomes and trichome attics may further contribute to such physical and spatial barriers. The trichome attic of *N. platani* may also reflect these selective factors as it has external walls that are thicker than normal, increases the distance to the immature beetle, and is filled with trichomes that may fool parasitoids accustomed to penetrating a single case wall to find their prey.

Faecal Case Evolution

Several leaf beetle lineages and perhaps as much as 20% of leaf beetle species cover their eggs or larvae in waste products to some degree (Vencl & Morton 1998; Vencl et al. 1999), although these are usually loose and structurally simple coverings. Camptosomate faecal cases may have evolved from such structures or may represent an independent origin of faecal architecture, reflecting this leaf beetle tendency. Well-developed cases have been found in ~45 Myo amber (Poinar 1999; Grimaldi & Engel 2005), indicating that they have long served their purpose(s). The selective factors driving initial case evolution may have involved ‘attempts’ to regulate such abiotic factors as temperature and humidity, or responses to biotic factors other than, or in addition to, the antipredatory functions demonstrated here. Further understanding the evolution of this complicated architectural/behavioural trait will require older, more morphologically intermediate faecal fossils and phylogenetic reconstruction of the faecal structures of extant leaf beetles. Also important are the effects of case evolution on the evolution of the immatures themselves. For example, the non-cryptic colouration, softness (highly reduced sclerotization), and seeming lack of chemical defences exhibited by *Neochlamisus* larvae could reflect the loss of ancestral defences following case evolution.

With respect to the function studied here, it is worth noting that different predators might select for different case traits. Thicker cases as well as rounder cases that are harder to grasp, could better thwart biting/chewing predators (as in Hoverman & Reylea 2007). Longer cases that enable larvae to retreat further from the case entrance might provide greater protection from bug beaks, parasitoid ovipositors, or other structures that reach into the case itself (as in Eisner et al 2002; Hoverman & Reylea 2007). Smaller case openings may better prevent entry by entire individual organisms. Indeed, big (relative to larval size), elongate cases with small openings (that can be plugged by the larval head capsule) occur in certain ground-dwelling camptosomate species, perhaps reflecting an evolutionary response to greater predation risk. Case shape evolution could also reflect various tradeoffs, such as those mentioned above. By contrast, adding trichomes to faecal cases might represent a comparatively cost-free means of increasing protection.

With respect to the species studied here, trichome attics may represent an efficient way to further protect a particularly vulnerable area of the case, and whose evolution may have been facilitated by preadaptation. Various camptosomate species seem to retain the original structure of the egg case as a "nipple" on the case apex (Flinte & Macêdo 2004; Reu & del-Claro 2004; Brown & Funk 2005). Two other genera of casebearers, *Exema* and *Lamprosoma*, are known to store shed larval skins or build empty chambers in the case apex, respectively (LeSage 1982; Erber 1988). These simple spaces may simply be nonadaptive byproducts of case construction that nonetheless provided the behavioural and architectural foundations for the future evolution of attic construction. Indeed, such simpler structures could serve similar roles to that of the attic in preventing predation. In fact, shed skins plus faecal shields proved more effective against predators than faecal shields alone in a tortoise beetle species (Müller 2002).

In sum, through detailed observation and experimentation, our study shows that the faecal cases of *Neochlamisus* larvae and pupae are sufficient to significantly lower the threat from diverse predaceous arthropod taxa. In addition, the extra architectural components of *N. platani* cases, i.e. external trichomes and trichome attics, were found to further this protective function. These results allude to possible reasons for which this complex behaviour may have evolved and possible pressures currently affecting its maintenance. Since other species of *Neochlamisus*, as well as other camptosomate taxa, live in ecologically divergent habitats, further studies could advantageously explore geographic and habitat-associated variation in case design and function. For instance, several *Neochlamisus* species live in the deserts of the southwestern United States and other casebearers live in close associated with ants (Erber 1988). These taxa may face particular constraints on case structure deriving from aridity or ant attack, respectively. At a finer scale, within-species geographic variation could reflect local adaptation to the varying suites of predators and parasitoids (or other factors, such as temperature and humidity) encountered, if these are consistent over evolutionary time scales. Such consistency appears plausible for *Neochlamisus* species, given their host plant and habitat specificity. Given our newly acquired understanding of case-associated predation resistance, future studies should investigate case traits across taxa, geography, and ecological contexts to further evaluate what specific selection pressures are promoting the evolution and variation of the amazing behavioural/architectural traits represented by camptosomate faecal cases.

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CHAPTER V

UNORTHODOX TASTES: ACTIVE APPROPRIATION OF AN ANTI-HERBIVORE DEFENSE BY TRICHOME-SEEKING *NEOCHLAMISUS* LEAF BEETLES

Abstract

Many plant species possess cellular hairs on the leaf cuticle (trichomes) that obstruct and repel arthropod herbivores . Prior work accordingly demonstrates that insects generally prefer leaves lacking such hairs. By contrast, this study identifies an herbivore that not only successfully negotiates these physical defenses, but actually seeks out hairier leaves, collects and co-opts their trichomes, and successfully develops on them, all to its own benefit. Specifically, it treats *Neochlamisus platani*, a leaf beetle specializing on American sycamore (*Platanus occidentalis*), a plant whose abaxial leaf surfaces possess low to very high trichome densities. Many of our experiments evaluate responses to densely trichome-covered (= pubescent) sycamore leaves versus those whose pubescence has been removed (= denuded) and/or those naturally having few trichomes (= glabrous). We found that although larvae feed equally on these three treatments, they consistently seek out and prefer to associate with pubescent foliage over other treatments. Field collections indicated that although females appear to oviposit on leaves without regard to trichome density, hatchling larvae subsequently locate and preferentially settle on highly pubescent foliage. Indeed, behavioral observations found that while young larvae may be slowed by trichomes, all instars are capable of removing them to facilitate feeding, and later instars often consume them. Performance tests illustrated the potential fitness benefits of such consumption, as larvae developed faster and larger on pubescent than denuded foliage. Immature stages of *N. platani* live within a “case” they fashion from their fecal material. These cases become externally covered with trichomes, and previous work shows that this covering contributes to protection from arthropod predators. Our rearing experiments suggested the active nature of such trichome incorporation as larvae deprived of pubescent foliage nearly always had fragments of filter paper (from their rearing dish) attached to their cases instead. Cumulatively, these results present *N. platani* as an insect that is behaviorally and physiologically adapted not simply to circumvent, but rather to actively prefer, use, and generally turn a primary physical defense of its host plant to its own advantage.

Key Words

animal architecture, direct and indirect effects, fecal cases, herbivory, host preference insect-plant interactions, leaf beetles, *Neochlamisus*, plant defenses, plant hairs, predation, pubescence

Introduction

Trichomes are “hair-like appendage[s] extending from the epidermis of aerial tissues” of plants (Levin 1973 p. 4). Diverse in structure, trichomes can serve a variety of functions, such as water exchange, protection from radiation, and storage of metabolic chemicals (Johnson 1975). However, trichomes may most commonly function to limit herbivory (Levin 1973). Herbivores can impose strong selection on plants, which are well known to possess numerous defensive mechanisms against their attack (Strong et al.

1984). For example, it has been frequently demonstrated that arthropod herbivores can be greatly limited in their ability to attack, survive, and develop on plants that are densely pubescent (e.g., Ranger and Hower 2002, Andres and Connor 2003, Valkama et al. 2005, Pelletier and Dutheil 2006). Nonglandular trichomes present a physical barrier that may slow or prevent arthropods from crawling on or reaching the leaf surface (Hoffman and McEvoy 1985, Baur et al. 1991, Medeiros and Moreira 2002). They can also entangle arthropods or pierce and kill them (Gilbert 1971). Even when able to feed on trichomes, herbivores are sometimes unable to digest them, thus reducing the nutritive quality of leaf tissues or even causing internal injury (e.g. Wellso 1973).

Despite the general effectiveness of trichomes as plant defenses, some insects have evolved behaviors and morphologies for moving and feeding on hairy surfaces (Johnson 1975, Southwood 1986, Bernays 1991), continuing the arms race between insect and host (Ehrlich and Raven 1964, Futuyma and Slatkin 1983, Berenbaum 1990). Insects bypass this barrier by cutting it down (Hulley 1988, Medeiros and Moreira 2005), spinning silk scaffolding over it (Rathke and Poole 1974), or evolving/developing longer legs and specialized claws to walk through it (Hoffman and McEvoy 1985, Moran 1986, Medeiros and Moreira 2002, Medeiros and Bolignon 2007). Herbivores may even enjoy some benefit from their host's trichomes if the hairs also impede their arthropod enemies (Gooderham et al. 1998, Michalska 2003, Roda et al. 2000, Koller et al. 2007, Lambert 2007). However trichomes are usually a net hindrance in the absence of predators (Guershon and Gerling 1999, 2001, 2006, Mulatu et al. 2004). And when given a choice, insects generally prefer glabrous leaves to pubescent ones when feeding or ovipositing (e.g., Baur et al. 1991, Woodman and Fernandes 1991, Zvereva et al. 1998, Ranger and

Hower 2002, Medeiros and Moreira 2005, Pelletier and Dutheil 2006). By contrast, few to no studies have demonstrated higher herbivore survival on or preference for pubescent leaves in the absence of predation risk.

Neochlamisus platani, an eastern North American leaf beetle, feeds exclusively on leaves from the American sycamore *Platanus occidentalis*. When young, these leaves are densely covered in short, branched trichomes, especially on their abaxial surface (Fig. 1). *Neochlamisus* belong to the leaf beetle lineage known as “casebearers”, with larvae that begin life as an egg within an individual “egg case” constructed by the female from her own fecal material. After hatching from this egg and creating an opening in the case, the larva continues to live within it, expanding it with its own fecal material as it grows, and eventually pupating inside it (Erber 1988, Brown and Funk 2005). *N. platani* cases are notable in that they tend, to varying degrees, to be covered in sycamore trichomes that adhere to it externally (Fig. 1A,B), and that are stored internally in an apical chamber of the case that is unique to this species, the "trichome attic" (Brown and Funk 2005). These case-associated trichomes have recently been shown to reduce the threat from biting/chewing predators (Brown and Funk *in review*). This finding raises questions involving the degree to which pubescent leaf usage and case-associated trichomes acquisition are active behavioral tendencies or incidental consequences and constraints associated with living on a plant with many pubescent leaves. More generally, it invites further investigation of the additional benefits and potential costs imposed by this lifestyle. For example, it has been hypothesized that the presence of trichomes in the

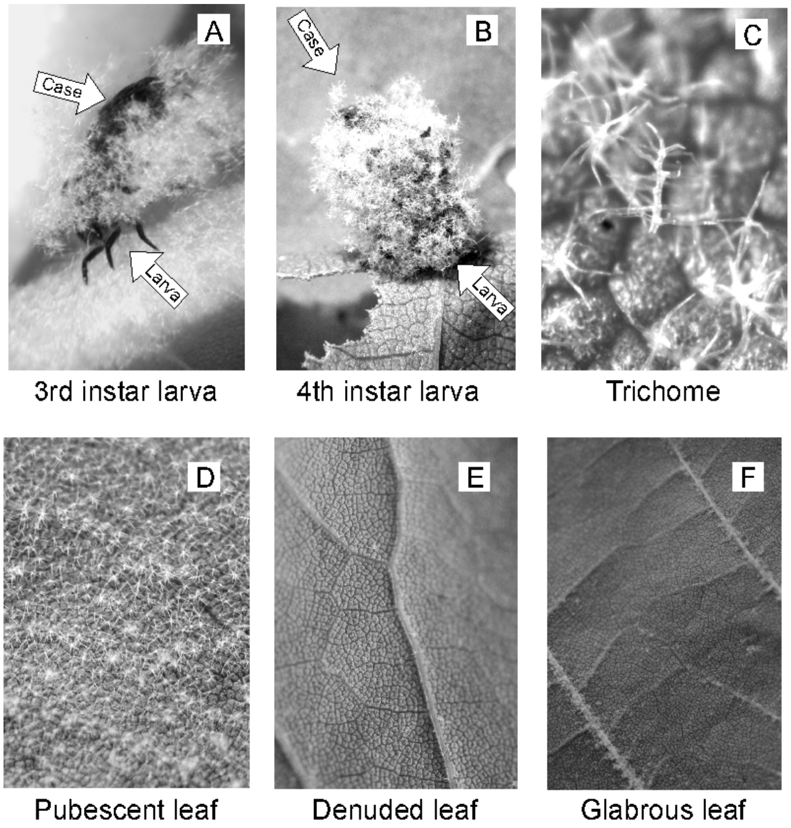

Figure 5-1. Photographs of *Neochlamisus platani* larvae and *Platanus occidentalis* (sycamore) trichomes, and leaf treatments. (A) 3rd instar larva with head and legs emerging from case opening. Note trichomes loosely attached to case. (B) 4th instar larva with denser trichome covering; case opening drawn close to the leaf surface. (C) The stellate (branched) trichomes on a sycamore leaf. (D-F) Leaf treatments presented to larvae in our experiments. (D) pubescent leaf = a trichome-covered leaf. (E) denuded leaf = formally pubescent leaf after removal of trichomes. (F) glabrous leaf = a leaf that naturally possesses no/few trichomes.

matrix of the case wall might strengthen it, furthering its protective function (Chaboo et al. 2008).

With these issues in mind, this study addresses a variety of trichome-related issues, including the following: (1) Do *N. platani* larvae prefer pubescent leaves or would they choose glabrous leaves if available, as in previous insect studies? If the former, are they attracted to the trichomes themselves or to some other aspect of the leaves on which they reside? (2) How do larval patterns of preference as a function of trichome density reflect patterns of larval and egg distribution on leaves in the field? What can be inferred about preference traits from these patterns? 3) What does close inspection of larval behavior reveal about how trichomes are dealt with and the degree to which they are actively manipulated? 4) What are the costs and benefits of larval development on pubescent versus glabrous foliage for these insects and their fecal cases? From the combined inferences derived from a variety of laboratory and field-based, manipulative and non-manipulative assays, this study reveals an herbivorous insect that not only bypasses its host's defense, but actively seeks them out, deals with them using specific behaviors, and enjoys fitness benefits associated with consuming them. In this paper, we document a rather unique example of an herbivore ecologically outmaneuvering its host plant.

Methods and Results

Study animals

Neochlamisus platani (Brown 1952) are small univoltine leaf beetles, which in middle Tennessee oviposit on sycamore in the spring and complete subsequent life stages on the host from the late spring to early summer, with larvae feeding on abaxial leaf surfaces. For this study, *N. platani* were collected on their host plants in the springs of 2006 and 2007 in and around Nashville, TN as adults, eggs, and larvae. These were maintained in plastic boxes lined with moistened paper towels in an incubator at 25 C and a 14:10 light: dark schedule at Vanderbilt University, Nashville, TN. Some boxes contained eggs kept en masse in Petri dishes lined with filter paper. These were checked daily for larval emergence. Boxes of larvae were either reared en masse on foliage or individually in stacks of 5 cm Petri dishes, each with a moistened filter paper and leaf cuttings. Boxes of adults were maintained en masse on small host branches to facilitate oviposition. Foliage, filter papers, and paper towels were regularly changed. This approach provided a constant source of test animals for the laboratory assays.

Study plants

The American sycamore *Platanus occidentalis* L. is a large deciduous tree that typically inhabits moist areas across much of the eastern United States (Nixon and Poole 2003). In the early spring, recently flushed, tender leaves (especially the abaxial surfaces, i.e., the ‘undersides’) are covered in short, stellate (i.e., branched) trichomes (Fig. 1C,E)

(Haines et al. 1985, Nixon and Poole 2003). Trichomes are only loosely attached to the leaf and when freed from it sometimes cling together, forming clumps on the leaf surface. Leaves lose trichomes (becoming more glabrous) and become tougher as they age (Nixon and Poole 2003). *P. occidentalis* trichomes are nonglandular and to our knowledge do not excrete any substances (KC Nixon, *personal communication*). Leaves of *P. occidentalis* used in experiments and for beetle maintenance were collected as needed from trees in the Nashville area. These were sealed in resealable plastic bags, transported in a cooler with icepacks to Vanderbilt University, and stored in a refrigerator until use.

Statistics

All data were analyzed using non-parametric methods due to deviations from normality. We used Kruskal-Wallis tests followed by Dunn's non-parametric means comparison test to determine differences between specific treatments for continuous variables (Dunn 1964, Zar 1996). Proportions were analyzed using a goodness of fit test (G) and a Bonferroni correction was applied when evaluating differences between multiple pairs of treatments. Case strength data were analyzed as paired comparisons using Wilcoxon's signed ranks tests for groups arranged as pairs (Sokal and Rohlf 1995). Means are presented ± standard errors, which we realize are not appropriate for non-normally distributed data, but we feel that these are most easily interpreted

Experimental leaf treatments

Larval preferences for most experiments were evaluated with respect to three ‘leaf treatments'. Leaves categorized as glabrous and pubescent were collected from one tree, usually adjacently on the same branches. “Glabrous” leaves (Fig. 1F) had few to no trichomes on the abaxial surface and were most likely older than those for the “pubescent” leaf treatment (Fig. 1D), that is, those that were densely covered in trichomes. The third treatment, "denuded" leaves (Fig. 1E), was produced by removing the trichomes from leaves that would otherwise be categorized as “pubescent”, by gently rubbing the leaf surface with a finger while wearing a nitrile glove. As foliage for the pubescent and denuded treatments used in a given replicate always derived from the left and right sides of the same leaf, these treatments were identical in every respect except the removal of trichomes. Larvae were presented foliage with the abaxial side up, on which feeding normally occurs in the field (Brown and Funk 2005). We avoided major leaf veins when cutting foliage for tests. No-choice tests and choice test 1 used leaf discs cut with a standard paper hole puncher, choice tests 2-4 used ~1cm^2 leaf cuttings, and choice test 5 used whole leaves. All tests except choice test 5 were conducted in 5cm Petri dishes lined with moistened filter paper. Sample sizes are in the figures.

No-choice feeding tests

To examine the willingness and ability of naïve hatchling *N. platani* larvae to feed on leaves of the three main treatments, each individual participated in three consecutive no-choice feeding trials that were performed in parallel fashion. In each trial an individual was given a single leaf disc from one of the treatments and allowed 24 h to feed. Feeding was then scored using a 20x20 ocular grid in a dissecting microscope at a magnification at which disc width equaled grid width. Specifically, the number of grid squares in which leaf material was missing from the dish was counted. After scoring, the

Petri dish was cleaned and the larva given a disk from another leaf treatment for trial two, and similarly for trial 3. The order in which leaf treatments were presented was systematically varied across larvae to avoid bias from prior exposure. Data for each treatment were analyzed as the average feeding score across individuals. Larvae with summed scores of < 10 were presumed to be unhealthy and their data excluded from analysis. *Results*: Larvae consumed all treatments nearly equally, showing no significant preference among treatments (Fig. 2A). Also worth noting is that the equivalent feeding on pubescent and denuded treatments supports the notion that our trichome removal did not release deterrent chemicals.

Repeated observation assays

Since larvae proved equally capable of feeding on each leaf treatment we performed a series of choice tests to evaluate whether other aspects of behavior might reveal patterns of preference for leaf characteristics that could explain why *N. platani* fecal cases are trichome-covered. These tests evaluated what traits larvae oriented to and how long it took for any preferences to be manifested. In the first four of these experiments, each larva was placed in the center of a Petri dish with moistened filter paper with three evenly spaced treatments and larval location recorded at regular time intervals. The fifth experiment was conducted similarly but at a larger and more natural scale (see below). Data for each treatment were analyzed as the percent of total observations involving that treatment, averaged across individuals. Observations of individuals on the arena rather than one of the treatments were excluded when calculating percentages. Choice tests 1 and 5 used only naïve hatchling larvae, whereas the others used larvae of various ages.

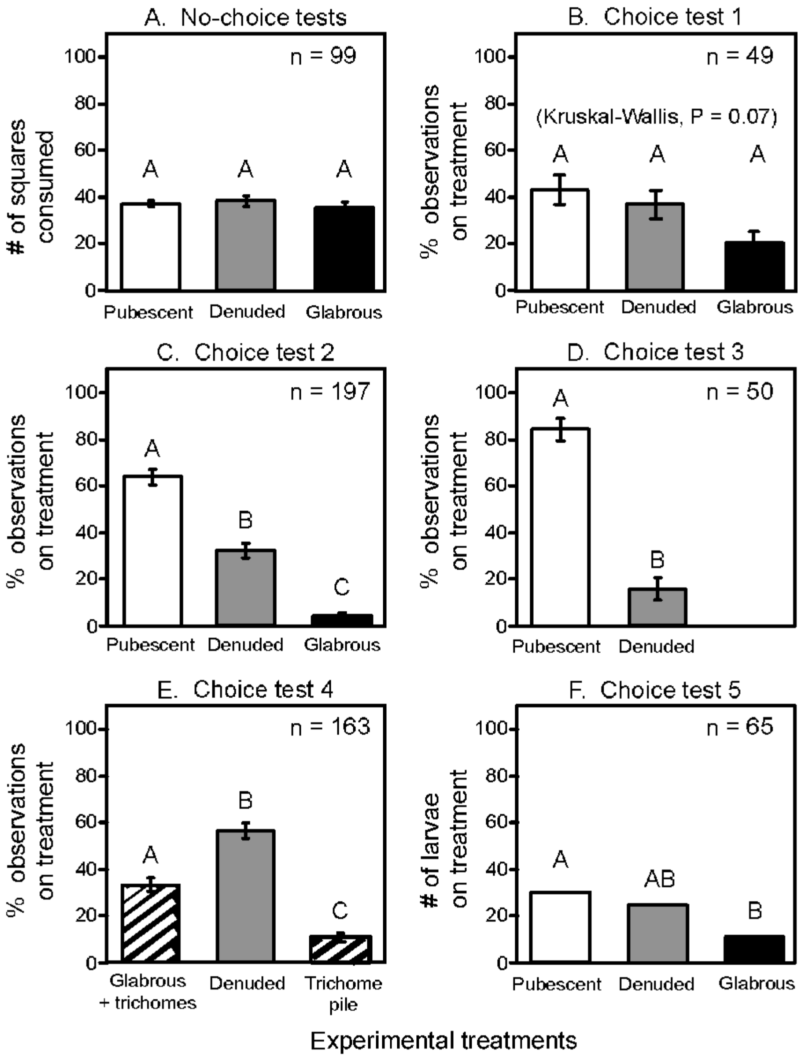

Figure 5-2. Results of feeding and location preference tests. Different letters within a plot indicate significant differences at P ≤ 0.05 using Kruskal-Wallace test followed by Dunn's non-parametric means comparison test. Means across individuals are presented ± s.e. (A) Amount of feeding in 24 h no-choice tests. (B - C) Percent of observations in a trial in which a larva was on cuttings of each treatment when observed: (B) every five minutes for one hour, and (C) every hour for five hours. (D) Percent of observations on pubescent and denuded halves of the same leaf cutting. (E) Percent of observations on glabrous leaf cuttings with loose trichomes placed on top, denuded leaf cuttings, and a pile of loose trichomes with no leaf. (F) Number of larvae on pubescent, glabrous, and denuded treatments represented by entire leaves, all attached to the same field-collected branch, after 24 hrs.

Data for some experiments are additionally evaluated with respect to change in patterns/preferences over the course of the experiment.

Choice test 1. Larvae (provided with the three main treatments were observed every 5 min for 1 h. *Results:* treatment differences were marginally non-significant (Kruskal-Wallis test, H = 5.4, df = 2, P = 0.07), although in the predicted order (Fig. 2B).

Choice test 2. The lack of significance in choice test 1, for data averaged across observation periods prior to analysis, belies a gradual increase in the percent of larvae on the pubescent treatment over time, yielding a significant preference to the glabrous treatment after 30 min (Fig. 3). This observation inspired a longer-term experiment in which larvae were given the same treatments and observed every hour for 5 h. *Results:* After the first hour, each pairwise treatment comparison was significantly and considerably different, with pubescent leaves used most and glabrous leaves almost entirely ignored (Fig. 3), Thus, the nonsignificant trend of time-averaged preferences observed in choice test 1 (Fig. 2B) became more pronounced and statistically significant in the present test (Fig. 2C).

Choice test 3. This experiment attempted to evaluate differences in larval preference for pubescent versus denuded treatments as precisely as possible using a highly controlled design. Larvae were presented with a single cutting of an initially entirely pubescent leaf, that had one side denuded of trichomes while the other was left unmanipulated. Larval location was then recorded every 30 min for 3 h as the previous test indicated this was sufficient time for larvae to exhibit preferences. *Results:* In 17% of total observations, larvae were located at the boundary separating the denuded from the pubescent sides of the leaf, usually feeding on the denuded side, while touching the

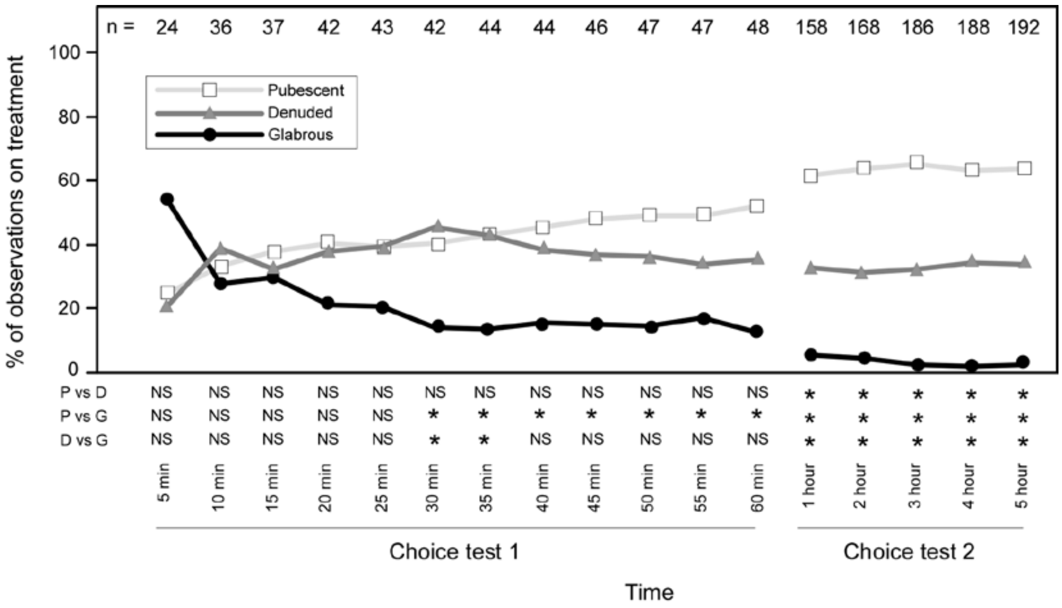

Figure 5-3. Time course of expression of preference for pubescent cuttings in choice tests 1 and 2. Percent of *N. platani* larvae observed on pubescent, glabrous, or denuded leaf cuttings for each observation period from choice tests 1 and 2 are depicted. Significant differences between treatments were determined using multiple goodness of fit tests (df = 2) with Bonferroni corrections. NS = not significant, * = significant at $P \leq 0.017$. Changing values of n reflect changing numbers of larvae observed on the arena rather than a treatment; these were excluded from analysis. Initial n = 53 for choice test 1; initial n = 200 for choice test 2.

trichomes on the pubescent side. These intriguing intermediate observations were excluded from further analysis. Of the remaining observations, > 80% were on the pubescent side. These highly significant results highlight how this design reveals a very strong preference of larvae for foliage with trichomes (Fig. 2D).

Choice test 4. In order to separate the preference for trichomes per se versus the preference for trichome-bearing foliage, larvae were given a choice between cuttings of glabrous leaves with a pile of removed trichomes placed on top, denuded leaves, and a pile of loose trichomes. Larvae were observed every hour for 3 h. *Results:* Larvae exhibited a significant preference hierarchy in the following order: denuded > glabrous + trichomes > trichomes alone (Fig. 2E). These results suggested that trichomes alone are not sufficient to elicit strong larval preference. However, the smaller difference between denuded and glabrous usage in this test compared to choice test 2 (Fig. 2,C), which was equal in duration, does suggest the that adding trichomes may have made glabrous foliage more acceptable. This supports the notion that trichomes per se do have some positive effect on preference.

Choice test 5. In this experiment larval preferences were tested under more natural conditions by observing larval localization on sycamore branches bearing three whole leaves, one representing each treatment, with the denuded treatment created by rubbing away trichomes from the entire abaxial leaf surface. At the initiation of each trial, multiple larvae were placed at the proximal end of the branch. The cases of these larvae had been individually marked with different colors of model airplane paint (Testors, Rockford, IL). Such marking has been used in a number of experiments and has no discernible affect on larval health or behavior (Brown and Funk 2005, *unpublished data*).

Each larva’s location (on a given leaf treatment, the branch, or the test arena) was observed every hour for 5 h and also after 24 h. Larvae found on the arena were repositioned on the proximal end of the branch. An average of ~3 larvae were added to each of 10 replicate boxes on each of three days, for a total of 30 replicate boxes and test 76 larvae. Each replicate received fresh foliage. *Results:* At our initial observation, the highest proportion of larvae were on the glabrous and the lowest proportion on the pubescent leaves, contrary to our hypothesis (Fig. 4A) However by the last observation the trend had reversed (Fig. 2F, 4A). There are three reasons to suspect that this experiment underestimates the true degree of preference. First, since glabrous leaves tend to be more proximately located on branches, treatment was strongly conflated with proximity to initial larval position (Fig. 4B) so the order in which treatments were encountered and evaluated by larvae moving along the branch was non- random (Fig. 4B). Since larvae tended to initially settle on the closest leaf (usually a glabrous one), this yielded biased preference estimates that took time to overcome. This period was lengthened by the 32 times that test larvae fell from the branch to the arena, thus being replaced near glabrous foliage once more and ‘resetting’ whatever progress they had made toward pubescent leaves. Third, data patterns changed dramatically at the 5 h observation, yielding a biologically incongruous pattern that would require many ‘pubescent’ and ‘glabrous’ larvae to switch positions. Removing this observation would yield curves that gradually and continually changed in the predicted directions. However, although it seems highly likely that some artifact or human error is responsible, we have no evidence for this and thus no legitimate reason to remove these data.

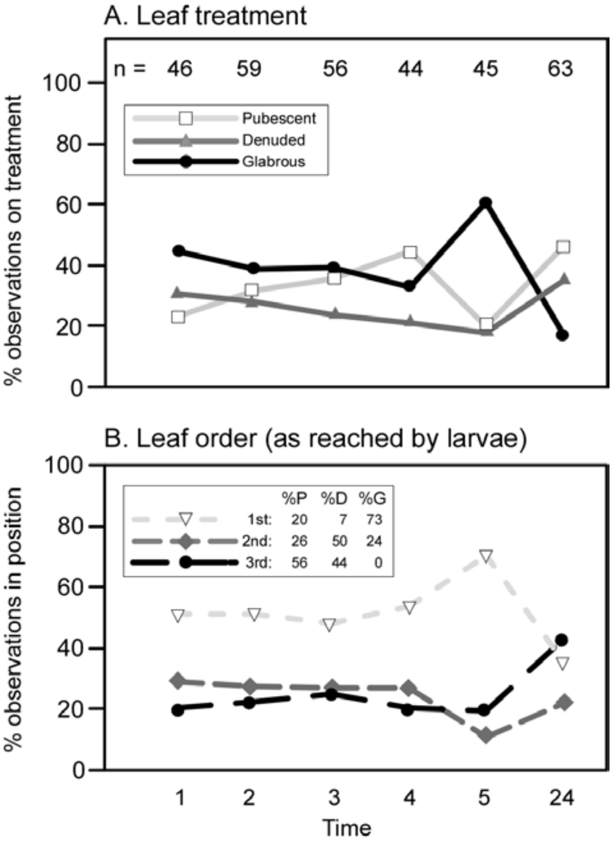

Figure 5-4. Time course of expression of preference for entire pubescent leaves despite confounding of treatment with leaf order on test branches in choice test 5. Larvae were initially placed at the proximal end of field-collected branches from which all but three entire leaves (representing treatments) had been removed. (A) Percent of larvae on each leaf treatment over time. (B). Percent of larvae on leaves as a function of distance from initial larval position (from 1^{st} to 3rd leaf on branch). Inset depicts distribution of leaf order as a function of treatment and reflects natural variation. Correspondingly, glabrous leaves were often encountered first and pubescent leaves last by test larvae. Patterns are thus conservative with respect to degree of larval preference for pubescent leaves. Changing values of n reflect changing numbers of larvae on a branch or the arena rather than a leaf at time of observation; initial n = 76.

Field observations

To evaluate whether the larval preference for pubescent foliage in our lab studies reflects natural distribution patterns, we collected young larvae and eggs from sycamore leaves in the field and evaluated the trichome densities of these leaves. To obtain a diverse sample, 30 trees from 13 sites in and around Davidson County, TN were sampled. To avoid biased searching, trees were visually scanned and whenever any egg or larva was observed, all leaves on that branch were carefully inspected and any additional leaves with eggs/larvae were collected. Specifically, upon collection we immediately cut a piece of leaf surrounding the egg/larva and placed it in a separate plastic vial, being careful not to disturb animal or trichomes. Vials were then immediately placed in an iced cooler for future transportation to the laboratory. Later on the day of collection, each egg/larva was removed from the leaf, its location marked with a pen, and each leaf assigned a random number. Leaves were stored overnight in a refrigerator and trichome density was estimated the following day in the absence of information on what life history stage had been collected from it. This was done using a dissecting microscope with its magnification set so that a 20 x 20 ocular grid represented a 5 x 5 mm square centered on the original location of the egg /larva. The number of grid squares including trichomes was then counted.

Leaf age is negatively correlated with trichome density (Nixon and Poole 2003), and the cases of inviable eggs can remain attached to leaves for a very long time (DJF *personal observation*). Thus, the distribution of eggs across leaves as a function of trichome density may not represent the distribution of leaves originally oviposited on, but be biased towards lower trichome densities by the inclusion of inviable eggs.

Accordingly, in an attempt to more accurately estimate female oviposition preference for pubescent leaves, field-collected eggs were individually maintained and daily checked to evaluate viability (as indicated by larval emergence). Eggs were characterized as inviable if they failed to produce a larva after a period greatly exceeding that normally required following oviposition (ca. 1.5 wks). This allowed the distributions of viable and inviable eggs to be separately evaluated. The distribution of larvae as a function of the trichome densities of their leaves was also evaluated.

Results: We found a total of 353 eggs and 108 larvae (mostly first instars) on leaves. Inviable eggs were found on leaves that were significantly less pubescent on average than viable eggs (Fig. 5), supporting the scenario described above. Intriguingly, larvae were found on significantly and considerably more pubescent leaves than were viable eggs (Fig. 5) (Kruskal-Wallis, $H = 94.0$, df = 2, $P < 0.0001$). Inspecting these distributions supports the hypothesis that females lay eggs rather indiscriminately with respect to pubescence levels, after which larvae preferentially seek out suitably pubescent foliage.

Behavioral Observations

Five *N. platani* larvae were observed continuously under a dissecting microscope to determine how they maneuver and feed on their host plant in relation to trichomes. One larva from each of the four instars (and two of the second instar) were observed. Each was starved overnight prior to observation to promote active feeding. Observations lasted 40.6 +/- 5.7 min per larva. *Results:* Larval age influences interactions with trichomes. First instar larvae are smaller than the clumps of trichomes that form on

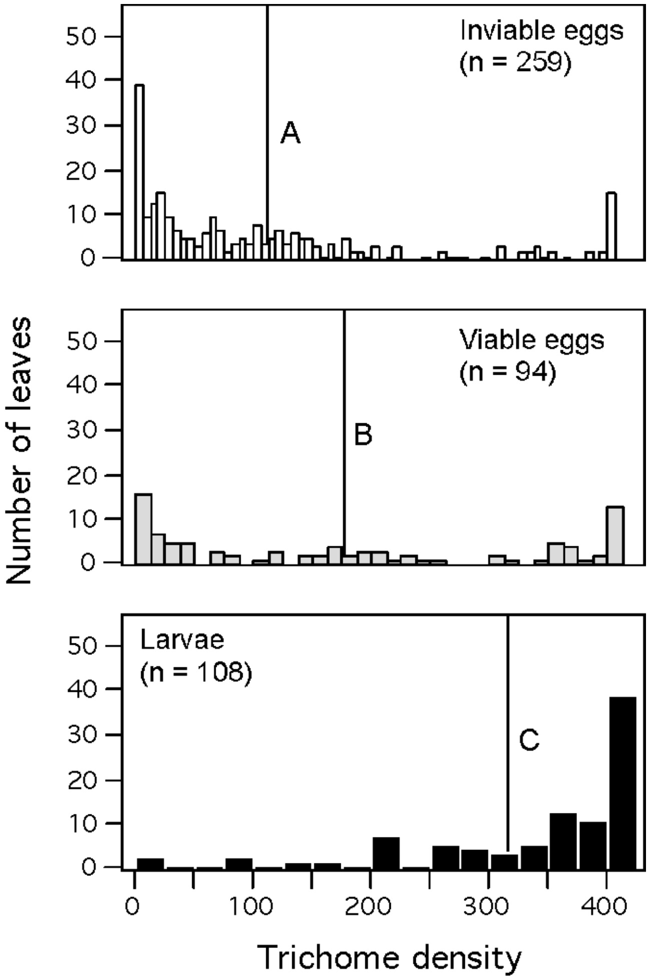

Figure 5-5. Distribution of the trichome density of leaves on which eggs and larvae were collected in the field. "Inviable" eggs are those from which a larva never emerged after collection. These (and their leaves) are expected to be older on average than viable eggs and the two types were thus evaluated separately (see text for details). Indeed, inviable eggs derived, on average, from less pubescent leaves (A vs. B), Most importantly, this plot demonstrates that ovipositing females and larvae differ considerably in degree of pubescent-based preference (B vs. C). Nonetheless, Different letters indicate significant differences in means (vertical lines) at $P \leq 0.05$ using Kruskal-Wallace test followed by Dunn's non-parametric means comparison test.

sycamore leaves and move through them with some difficulty. A second instar larva walked through a divide in the trichomes it created by waving its forelimbs. Some early instar larvae walked on top of the trichomes, above the leaf surface, while others used individual trichomes as footholds to anchor their movement closer to the leaf surface. The fourth instar larva was much larger than the trichomes and readily walked among them, digging its tarsal claws directly into leaf tissue. All instars clipped or pulled trichomes loose by pinching the trichome base with their mandibles and then tossed them to the side and out of their path (Fig. 6), as seen previously in this species (Brown and Funk 2005) and some other insects (Hulley 1988, Medeiros and Moreira 2005). None appeared to deliberately attach loose trichomes to the wet fecal material recently added to the base of the case as part of case enlargement activities. Nonetheless, loose trichomes readily cling to such material and to trichomes already attached to the case when they come in contact. Larvae were observed to clear a local area of trichomes, especially when feeding, which this behavior presumably facilitates. The large 4th instar larva did not exhibit this behavior, instead appearing to consume the trichomes along with the rest of the leaf material. Subsequent dissection of 27 4th instar larvae recovered identifiably complete, branched trichomes from the gut of 17, confirming this behavior. Given these behaviors it is clear how trichomes come to be incorporated in and on larval fecal cases even if larvae do not have behaviors directed at ‘actively’ adding trichomes.

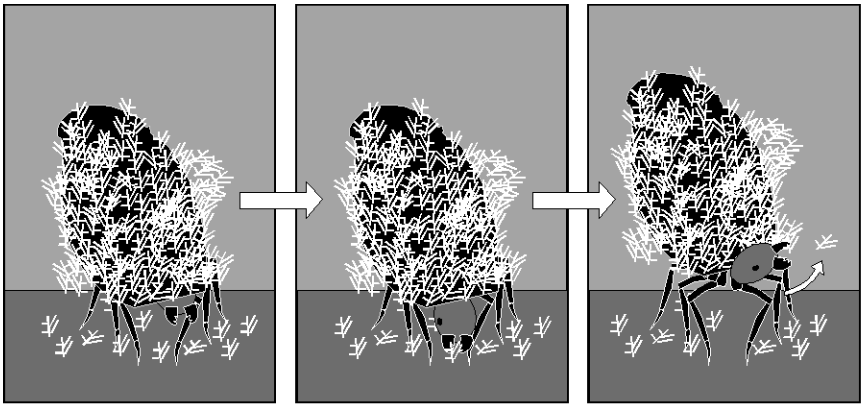

Figure 5-6. Diagrammatic representation of a 4th instar *N. platani* larva clipping trichomes with its mandibles and removing them from its feeding area by tossing them aside with a flick of the head.

Effect of trichomes on development and case strength

Fecal cases are known to resist attacks from various arthropod predators (Wallace 1970, Root and Messina 1983, Brown and Funk, *in review*). The stronger the case, the longer it takes biting/chewing predators to break through and reach the immature beetle inside (Brown and Funk, *in review*). SEM work has shown that the matrix constituting *Neochlamisus* larval case walls contains trichomes and thus may represent a composite material stronger than walls composed of feces alone (Chaboo et al. 2008). Such material might be compared to reinforced concrete (Hansell 2005). We tested whether or not trichome availability affects case strength by rearing pairs of larvae from emergence to pupation ad controlling trichome availability. In each pair, one individual was reared entirely on pubescent leaf cuttings while the other received only denuded cuttings from the same leaf. After pupation, all cases were maintained for 17 days at constant temperature and humidity to standardize abiotic effects on case strength. The proportion of case covered with trichomes for each case was estimated visually using a subjective scale of 0-5 (0 = no trichomes on case and 5 = 100% coverage). Case strength was measured as the force required to crack the case wall, using a penetrometer mounted on a moveable slide and pulled toward the case with a crank-and-pulley system (see Brown and Funk, *in review* for more details).

When making observations on trichome density we noticed that individuals reared on denuded leaves tended to have other debris attached to their case (Fig. 7A). This debris consisted of leaf fragments, shed skins, and loose balls of fecal material, but most often consisted of fibers from the filter paper lining the Petri dish in which they were reared. Therefore each individual was also separately scored for the presence of filter

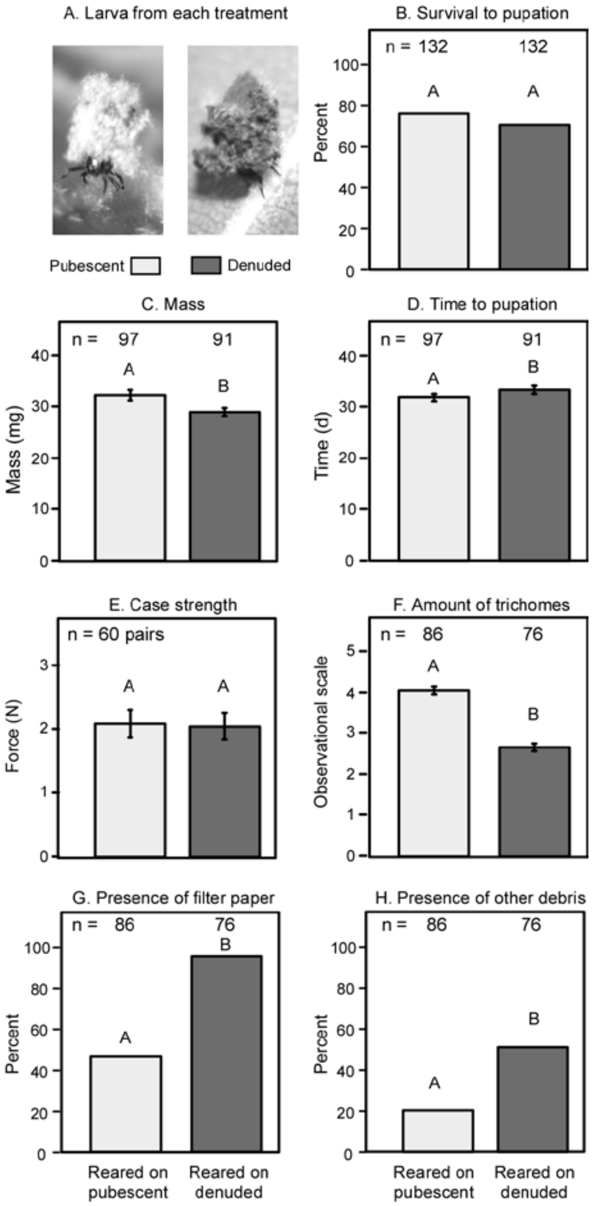

Figure 5-7. Results from larvae reared on pubescent versus denuded leaves . Different letters within a panel indicate significant differences at $P \leq 0.05$ using Kruskal-Wallace test for continuous measurements, Goodness of fit test for proportions, and Wilcoxon's signed ranks tests for groups arranged as pairs for case strength; means ± s.e.. (A) Typical third-instar larvae from each treatment. Note their trichome-covered versus debris-covered cases. (B) Percent of larvae surviving to pupation. (C). Mass of larvae immediately prior to pupation. (D) Time between larval ‘emergence’ from the egg case and sealing the case for pupation. (E) Force required to break case wall with a penetrometer. (F) Amount of trichomes on cases using a subjective scale, in which: 0 = no trichomes and 5 = 100% coverage. (G) Percent of larvae with filter paper fragments on cases. (G) Percent of larvae with other forms of debris (i.e., not including filter paper or trichomes) on cases.

paper debris and of other debris. We also recorded basic performance measures for the two treatments, specifically time until pupation, survival to pupation, and mass at pupation.

Results: We found no effect of rearing treatment on survival to pupation (Fig. 7B), but larvae from the pubescent treatment animals attained a significantly larger pupal mass (Fig. 7C) in less time (Fig. 7D). Since larvae were weighed with their cases, this difference in mass might be due to the weight of case-associated trichomes. To evaluate this, we collected mature fourth instar larvae in the field, removed the trichomes from the exterior of their cases, and weighed them. These weighed 0.84 +/- 0.06 mg (n = 5), accounting for an average of 3.10% of total mass (i.e., of larva + case + trichomes). By comparison, the mean mass difference of our experimental rearing treatments was 3.3 +/- 1.19 mg on average or ~10% of total mass. Thus, external trichomes cannot account for the greater mass of larvae reared on pubescent foliage.

We found no differences in case strength between the two treatments (Fig. 7E) despite the greater abundance of external trichomes (and likely increase in wall-matrix trichomes) for the pubescence-reared treatment. (Fig. 7F). Interestingly, cases from individuals reared on denuded leaves did prove significantly more likely to possess filter paper (Fig. 7G) and other debris (Fig. 7H) than those reared on pubescent leaves. This finding is consistent with the hypothesis that larvae do have an innate tendency to manipulate their substrates such that it somehow becomes attached to their cases.

Discussion

General findings

Trichomes are a common physical defense used by plants to prevent successful herbivory, and their presence on leaf surfaces greatly affects the abundance, efficiency, and fitness of arthropods, usually quite negatively (Levin 1973, Southwood 1986). In most studies, insects prefer to feed on foliage from which the trichomes have been removed. Here, we show that unlike most insects, *Neochlamisus platani* larvae are not only capable of feeding on pubescent foliage equally as well as denuded and naturally glabrous leaves, but also actively seek out leaves with denser trichomes, both in the laboratory and field. Larvae preferred pubescent leaves over glabrous ones in 4/5 choice tests, even when treatments derived from the same leaf. In the fifth test, adding trichomes to naturally glabrous leaves increased the acceptance of larvae for this leaf type, further demonstrating that trichomes per se (and not just trichome-bearing foliage) contribute to larval preference. In the field, viable (younger) eggs were found on significantly more pubescent foliage than inviable (older) eggs, yet even viable eggs were found on leaves representing a broad range of trichome density. By contrast, larvae were almost never found on leaves with lower trichome density. These results support the notion that females lay eggs indiscriminately in terms of leaf pubescence (and its correlate, age) and only later do larvae actively migrate to young pubescent leaves for feeding and development.

We found no evidence that trichomes hinder *N. platani* larvae to any biologically-significant degree, despite some loss of maneuverability for younger larvae. In fact, when reared on pubescent foliage, larvae were quicker to reach pupation and did so at a larger mass, than when reared on trichome-free foliage. Larvae of all ages were able to remove trichomes by clipping them, while older larvae actually ate the trichomes. These behaviors are, respectively, likely to help account for the observation that trichomes become externally attached to, and incorporated in the walls of, fecal cases in this species. Indeed, larvae seem hardwired to incorporate materials from the substrate into their cases, as evidenced by the external incorporation of various forms of debris in the cases of larvae deprived of access to trichomes. This tendency may reflect the effectiveness of trichomes at reducing predation risk (Brown and Funk, *in review*). Contrarily, we found no effect of trichome accessibility on case strength in this study. Below, we explore these and other possible benefits *N. platani* may receive by seeking out densely pubescent foliage, an example of an insect with truly unorthodox tastes.

Possible benefits: indirect

Larval *N. platani* may benefit from their choice to use pubescent leaves for several reasons, both direct and indirect. They may benefit indirectly because trichomes deter their competitors or predators. As with other plant defenses, other insect species might conceivably become specialized to deal with trichomes as *N. platani* has (Smith and Kreitner 1983). However, our own field experiences suggest that there may be few such herbivorous sycamore specialists to compete with *N. platani*. More concrete evidence is provided by Thompson and Solomon (1986), who found only "light populations of 10 species of defoliators" in sycamore plantations. Other studies report finding fewer individuals of fewer beetle species (Ulyshen and Hanula 2007) and lower feeding rates (Morewood et al. 2004) on sycamores when compared to co-occurring tree species. Thus, perhaps the heavily pubescent leaves of *Platanus occidentalis* do help these plants successfully resist exploitation by insect herbivores. However, another study found sycamores to have lower nitrogen concentrations than the three other tree species studied, even following fertilization (Chang and Robison 2003). As nitrogen content can be positively associated with herbivore growth and survival (Mattson 1980) trichomes alone may not account for their relatively herbivore-free status.

Trichomes can also entangle and deter arthropod predators of herbivorous species (Gooderham et al. 1998, Roda et al. 2000, Koller et al. 2007, Lambert 2007) or hide herbivores feeding beneath them from visual predators (Bilderback and Mattson 1977, Michalska 2003). However, these benefits are generally associated with costs in the absence of predators. For instance, insects may have shorter lifespans (Guershon and Gerling 1999, 2001, 2006) or lower fitness (Mulatu et al. 2004) when reared on pubescent leaves as compared with glabrous leaves. However, we found no such discernable costs to feeding on pubescent leaves for *N. platani,* but rather associated increases in fitness (see below). Therefore, *N. platani* could also experience a net benefit from the trichome-restricted movement of their predators or parasitoids. In what might provide an extreme illustration of this, very young *N. platani* larvae tend to nestle themselves tightly in the basal forks of the major leaf veins, where trichomes are particularly dense, a behavior that is not exhibited by any other *Neochlamisus* species. In this position, larvae are likely nearly inaccessible to and well defended from most predators. Further, our observation that inviable eggs were found on less densely pubescent foliage than viable eggs, may not simply reflect greater age of eggs and associated leaves. The failure of these eggs to produce larvae was frequently due to their being parasitized. Thus, this pattern could also reflect a tendency of trichomes to reduce the efficiency of *Neochlamisus* egg parasitoids. This hypothesis is supported by the fact that inviable eggs yielding wasps were found on less pubescent foliage (86 ± 10.0, n = 109), than the other inviable eggs (133 ± 10.4, n = 150) (Kruskal-Wallis, $H = 10.9$, df = 1, P = 0.001). However, this possibility begs the question of why females do not restrict themselves to ovipositing on highly pubescent foliage, rather than exhibiting the haphazard pattern documented here? Perhaps female movement and egg-case building and attachment behaviors are also appreciably limited by trichomes.

Again, recognizing the inverse relationship between degree of pubescence and leaf age, consider that several of our experiments show larvae to be more attracted to previously pubescent foliage that has been denuded of trichomes than to hairless glabrous foliage. Thus, larvae appear not only to be attracted to trichomes alone, but also to younger foliage per se. Younger leaves typically provide herbivores with higher nutrition levels, which is why they are often highly defended, usually chemically, but also with high-density trichomes (Feeny 1996, Coley 1980, Choong 1996, Read et al. 2003). By contrast, older glabrous leaves are likely to contain fewer proteins and more tannins and thus are often less nutritious by comparison (Feeny 1970, Choong 1996, Read et al. 2003). Therefore *N. platani* may also be selected to associate with trichomes as they provide a cue to better quality food (Southwood 1986, Read et al. 2003). Such nutritional benefits of feeding on younger leaves may outweigh any negative effects of mowing through or eating the trichomes (e.g., Wellso 1973).

Possible benefits: direct

N. platani also directly benefit from the trichomes of their host plants. For example, we show here that these trichomes are eaten by larvae. If they are digested, they may directly provide larvae with additional nutrients (Knutson 1982, Smith and Kreitner 1983, Wellso 1973). We know nothing of the nutritional value of *P. occidentalis* trichomes, but when reared on pubescent versus denuded leaves, larvae reached pupation faster and achieved greater mass. Larger larvae produce larger adults, which tend to have higher rates of survival and fecundity (Honek 1993, Kingsolver and Huey 2008). Developing at a faster rate has also been shown to increase survival by reducing exposure to predators (e.g., Bernays 1997). Thus, trichome feeding could itself increase fitness.

Most impressively, trichomes protect *N. platani* from arthropod predators by contributing to a physical defense. *N. platani* larvae are casebearers and thus live in and maintain a case of their own waste material. Unlike most other casebearers, *N. platani* cases become very fuzzy as trichomes are incorporated into and onto the case wall (Brown and Funk 2005, Chaboo et al. 2008). *N. platani* also bring trichomes into the case and seal them off in the apex with a layer of feces, creating a “trichome attic.” Although we did not find here that trichome availability strengthened the case wall, external trichomes and attics do increase survival, lower the threat of attack, and aid larval escape from arthropod predators (Brown and Funk *in review*).

Trichome incorporation may occur as loose hairs get stuck in the wet feces added to enlarge the case, through incomplete digestion of plant hairs, or by larvae actively collecting hairs and inserting them into the case material. Although we did not observe deliberate trichome insertion, trichome "mowing" and tossing was a common occurrence and could account for much of the trichomes ultimately attached to the exterior of the case. Indeed, *N. platani* larval behavior seems to necessarily result in the incorporation of material from their environments into and onto the case wall. This was apparent from our observations that when trichomes were unavailable, fecal pellets, leaf shards, and filter paper fragments were often found on cases instead. Again, much of this debris may have been passively accumulated. However, in this context the relative lack of such debris in the cases of larvae reared on pubescent foliage is intriguing. This contrast seems to indicate either that the presence of external trichomes prevents other substances from clinging to the case, or that these larvae are hardwired to actively incorporate material from their substrates into their cases. In particular, filter paper is rather tough and fragments have not ordinarily been observed to appear during our lab’s normal rearing of immature *Neochlamisus*. This, and the experimental prevalence of such fragments described above, suggests the likelihood that these fragments were actively scraped off. Other species in the casebearing lineage of leaf beetles build their cases out of quite a variety of materials other than feces, including abiotic materials and artificial substances (when made available) (Erber 1988), so perhaps *N. platani*’s indiscriminately acquisitive behaviors are somewhat atavistic.

Exceedingly few herbivores are known to benefit directly from the trichomes of their hosts in any manner, a very different kind of example being provided by a mite that feeds on pollen and fungal spores trapped in the trichomes of its primary host (Roda et al. 2003). Southwood (1986) suggests other possible positive associations between trichomes and insect fitness, including host-finding by released olfactants (in glandular trichomes), providing a literal foothold, limiting the efficacy of predators and pathogens, avoiding insecticides, and creating favorable microclimates (e.g., Horgan et al. 2007). However, as mentioned above, these benefits so far seem generally to come with costs. By contrast, we have as yet identified not cost to *N. platani* of its active and successful co-option and general exploitation of its hosts seemingly otherwise successful physical defenses.

Conclusion

The lives of insect herbivores are intimately tied to the surfaces of plants (Southwood 1986) and plants evolve traits in response to insect herbivores, furthering coevolution that has lasted hundreds of millions of years (Scott et al. 1992). Plants commonly respond to herbivores through the production of toxins, and the sequestration of a host plant's chemical defenses is widely found among insects, defending them from their enemies (Duffey 1980). Confiscating and using a host's chemical barriers can be costly, entailing the evolution of complex digestive systems and storage/secretion glands or requiring injury to be effective (Duffey 1980). Insects that bypass their host's physical defenses, such as leaf toughness, smoothness, or hairs, likewise typically require specialized (in this case morphological) adaptations, such as robust jaws, extended legs, and claw modifications (e.g., Karban and Agrawal 2002). *N. platani* larvae, by contrast, overcome and co-opt their hosts physical defenses without obvious morphological modification to themselves, but via behavioral adaptations leading to the "sequestration" of host trichomes in their cases. Our study thus provides a unique example of an herbivore that seemingly suffers no negative effects of the plant's hairy defenses, but rather co-opts it and gets away with it. The above discussion points out a variety of future directions to further our understanding of the ecological, evolutionary, behavioral, and physiological aspects of this unique insect-plant interaction.

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CHAPTER VI

HOW MOIST SHOULD FECES BE? ECOLOGICAL TRADEOFFS IN A PHENOTYPICALLY PLASTIC ANIMAL ARCHITECTURE

Abstract

Whether a given trait is beneficial or costly can depend on its potentially contrasting affects on different functions or in different contexts, yielding tradeoffs that are subject to selection. Phenotypically plastic traits that vary as a function of induction across environmental contexts can generate quite intriguing ecological tradeoffs when the cost/benefit of a given trait with two different functions actually switches in different environments. In our study, we documented this kind of tradeoff while testing hypotheses on the functional significance of a unique animal architecture. Specifically, we studied the effects of humidity/moisture on immature *Neochlamisus* leaf beetles and, especially, on the portable fecal cases that they construct and live within. These studies provided no evidence that the case reduces larval drowning when submerged, although it did increase floating versus sinking. Nor did cases reduce mass change (e.g., due to desiccation) or increase maturation and survival of pupae maintained at three humidity levels. However, we showed that when pupal cases were maintained at high humidities, case strength was weakened, such that adults successfully emerged from their pupal fecal cases but proved more susceptible to cricket predation. By contrast, pupal cases maintained at low humidities were of considerable strength, preventing adults from cutting themselves free, but proved more resistant to cricket attack. Thus we discovered that these cases impose a fundamental tradeoff with respect to two fitness-associated factors: adult emergence versus predator resistance. More intriguingly, we show that the direction of this tradeoff changes as phenotypically plastic consequence of varying humidity. We discuss the implications of these findings for *Neochlamisus* ecology and evolution, and the role of different sorts of tradeoffs for understanding animal architectures.

Key Words

building behaviors; costs and benefits; defense; drowning; ecological constraints; fecal cases; *Neochlamisus* leaf beetles; plasticity; predation; tradeoffs

Introduction

Animals from a variety of taxa build elaborate structures of disparate design and material make-up (von Frisch 1974, Hansell 2005). Such animal architecture can ameliorate adverse conditions by increasing food supply, reducing predation, or limiting the effects of dryness, heat, cold, or mechanical damage (Danks 2000, 2002, 2004, Hansell 2005). Spider webs, bird nests, and beaver lodges are well-known examples. Much work has evaluated the specific functions served by particular animal architectures (Hansell 2005). It is rather seldom shown, however, how varying ecological conditions may affect architecture structure and function (e.g., Jones et al. 2002).

A genetically based trait may affect different components of individual fitness in opposing directions via its differential effects on alternative functions. Such tradeoffs are classically illustrated by the example of the male peacock, whose majestic feathers no doubt benefit it through reproductive success, while imposing a cost through its likely negative effects on survival (via reduced predator evasion). Phenotypically plastic traits, in which the environmental influences the phenotype, can impart tradeoffs that are intriguing because they are ecologically dynamic (Padilla and Adolph 1996, DeWitt et al. 1998). Among these, behavioral traits, which may respond quickly and reversibly to changing conditions, might often be less costly than morphologically based plastic traits (Sih 2004). Animal architectures fall somewhere in between as they are physical manifestations of specific behaviors and may be subject to continual alteration, both by their builder’s behavior and by local ecological conditions. Thus, one might re-evaluate Pigliucci's (1996) boundaries on "how organisms respond to environmental changes" to include architecture as part of the extended phenotype.

Species in one group of leaf beetles, the Camptosomata, build a striking example of animal architecture out of their own feces, and continue to elaborate it during immature development. Briefly, each oviposited egg is surrounded by fecal plates by the mother, ultimately forming a sealed “egg case”. The hatchling larva cuts away the top of this case, allowing its abdomen to remain within the case while its legs and head can emerge from this case opening (Fig. 1a) (Erber 1988). The larvae carries this case and enlarges it with its own fecal material during its development before finally sealing closed the opening of the "larval case" and fixing it to the substrate to create a "pupal case," within which it metamorphoses into an adult (Fig. 1b). The mature adult then cuts away the apex of the case to escape (Fig. 1c) (see summary of case-associated construction and life history in Erber 1988, Brown and Funk 2005). Fecal cases are known to provide a partial physical barrier to predators (Wallace 1970, Root and Messina 1983, Brown and Funk in review), but no one has examined case function with respect to any abiotic condition, such as low humidity. Avoiding desiccation is one of the major adaptive hurdles faced by insects that feed on plants (Strong et al. 1984). And it has been hypothesized that fecal cases might mitigate the effects of harsh abiotic conditions; especially low humidity (Karren 1964, 1972, Boldt and White 1992, Olmstead 1994, Flinte and Macêdo 2004, Chaboo et al. 2008). In fact, larval camptosomates possess a spiracular structure (for oxygen intake) that inefficiently retains moisture (Moldenke 1971, LeSage 1982, Root and Messina 1983), while larvae removed from their cases tend to become lethargic and generally do not survive long (Karren 1964, Erber 1988, Flinte and Macêdo 2004, Brown and Funk 2005, *unpublished manuscript*, *personal observations*).

Here, we examine the effects of varying humidity/moisture conditions on fecal cases and associated aspects of insect fitness in the camptosomate *Neochlamisus*. Specifically we evaluate (1) effects of case presence/absence (hereafter "case effects") on larval survival upon immersion in water, (2) case effects on pupal mass across varying relative humidity (RH) conditions, (3) case effects on pupal survival and adult emergence as a function of RH, (4) case strength as a function of RH, and (5) effects of case strength on predator handling time. Our experiments led to the unanticipated discovery of an ecological fitness tradeoff caused by phenotypically plastic changes in a morphological product of behavior (the case) when exposed to disparate RH. This tradeoff is clearly of considerably biological relevance, with important consequences for fitness, given the ecology of these beetles. We interpret our findings in light of *Neochlamisus* biology and

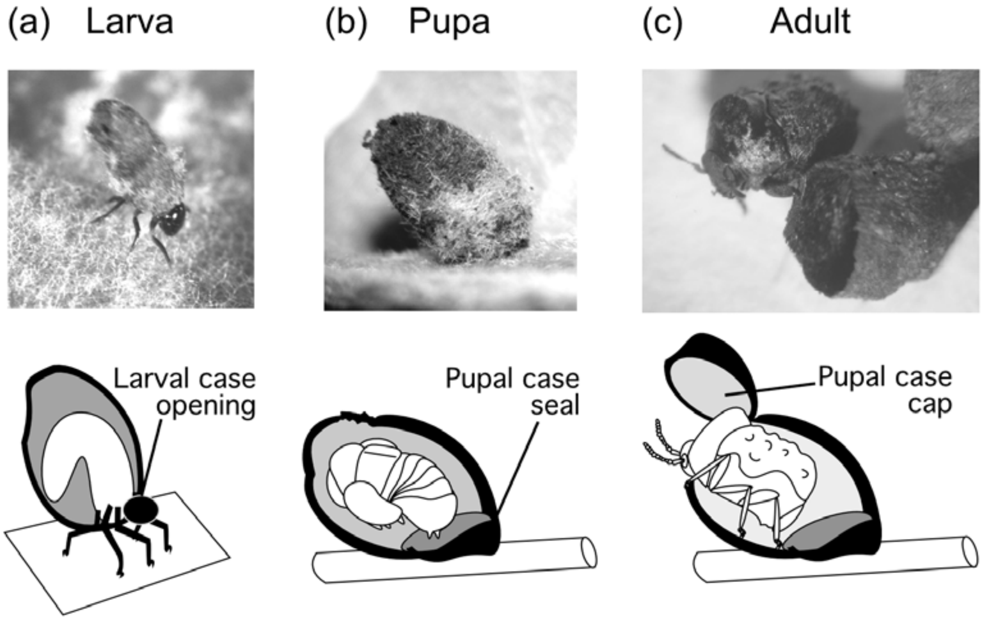

Figure 6-1. Photographs and cut-away diagrams of fecal cases containing (a) a larva, (b) a pupa, and (c) an emerging adult. Figure modified in part from Brown and Funk 2005.

discuss their relevance for understanding the ecology and evolution of this intriguing example of animal architecture.

Methods

Study organisms and test conditions

Neochlamisus Karren (1972) are ca. 4mm-long North American camptosomate leaf beetles (Chrysomelidae: Cryptocephaline). This study treats individuals of *N. platani* (Brown) and *N. bebbianae* (Brown), two closely related and possibly conspecific (Funk, *unpublished data*) species, that were collected from field sites in Nashville, TN, and northeastern North America, respectively. Test animals were reared from larvae collected in the field and those deriving from the lab-laid eggs of field-collected adults. This was done in incubators at 24C and a 14:10 light:dark cycle, primarily in sealed plastic shoeboxes lined with moistened paper and host plant foliage. In all experiments, test animals of appropriate life stage were haphazardly divided into ‘cased’ and ‘caseless’ and three RH treatments. Caseless pupae were obtained by opening the base of the sealed pupal case with a razorblade and removing the pupa. This procedure is harmless to the animals, which are not physically attached to their cases (Brown and Funk 2005). All but the submersion tests evaluated effects on test animals and their fecal cases of ‘low’ (≤ 30%), ‘medium’ (65-75%) and ‘high’ (≥95%) relative humidity (RH) conditions maintained in separate incubators. Low RH was achieved using the incubator’s dehumidify option. Placing a 5-gallon digitally programmable humidifier in the incubator yielded medium RH conditions. Individuals in these treatments were maintained in Petri dishes lined with dry filter papers in open plastic boxes. High RH was reached by placing pupae in tightly sealed plastic boxes lined with greatly moistened paper towels. For all treatments, RH was regularly monitored via a digital hygrometer within each incubator. Sample sizes are indicated in the figures.

Statistical Analyses

All primary data were analyzed using non-parametric methods due to deviations from normality. Kruskal-Wallis tests (H) were generally followed by Dunn's means comparison tests to inspect RH treatment differences (Dunn 1964, Zar 1996). Proportions were analyzed using Goodness of fit tests and G tests of independence (G). Box plots depict the 10th, 25th, 75th, and 90th percentiles and medians.

Effects of case on surviving submersion

Species of *Neochlamisus* often live on plants found in and near lowland wetlands (Karren 1972, Petrides 1998) that fluctuate greatly in water levels, and into which larvae may fall. Thus we tested case effects on larval submersion in water. Specifically, we submerged larvae in distilled water at room temperature (23-24C) for experimental trials of increasing duration. To ensure full submersion, larvae were placed individually in 85 mL plastic cups that were overturned within, and placed on the bottom of, plastic shoeboxes filled with distilled water. A penny placed on top of each cup prevented floating. Submerged cups contained no air. Larvae were randomly assigned to cased and caseless treatments with respect to mass, which did not differ between treatments (H_1 = 0.15, P = 0.70). Successive trials lasted 0.25, 1, 2, 4, 8, 16, 32, and 64 consecutive hours.

After each trial, test larvae were placed in Petri dishes with dry filter paper and checked for movement. Death was assumed if movement did not occur within one hour. Live larvae were then tested in the next trial. Following the 0.25hr and 2hr trials, larvae were used in the next trial ~1.5hrs after the previous one. After all other trials, surviving larvae were put in dishes with their host plant and maintained overnight in an incubator at 24C prior to participating in the next trial. Observations during early trials prompted us to further note if, after one hour, larvae were floating at the top of the cup or lying on the bottom.

Effects of case on pupal mass change as a function of RH

To test the hypothesis that *Neochlamisus* fecal cases can affect pupal mass (e.g. by resisting desiccation), we exposed cased and caseless pupae to the three RH conditions for five days. All pupae were then weighed (without cases) using a digital balance (Mettler-Toledo MX5, Columbus, OH). Since cases are destroyed when pupae are removed, we could not obtain caseless pupal mass from cased individuals at the initiation of trials in order to determine individual-level mass change during the trial period, so treatment-level comparisons were made instead. On this point it is relevant that individuals assigned to cased versus caseless treatments did not differ in mass (i.e., prior to case removal, for future ‘caseless’ individuals) ($H_1 = 0.0003$, P = 0.99); nor did those assigned to different RH treatments ($H_2 = 1.41$, P = 0.49). There were also no differences in pupal ‘age’ (i.e., time since the case was sealed for pupation) among case treatments ($H_1 = 0.44$, P = 0.51) or RH treatments ($H_2 = 1.23$, P = 0.54).

Effects of case on survival and adult emergence as a function of RH

In this experiment, pupae from each treatment were placed in incubators and daily monitored until either adults were observed or individuals were considered dead. For caseless individuals, survival to adult status was recorded when the pupal skin was shed, yielding a teneral adult; for cased individuals, this was recorded when an adult emerged from its case. Death was established by the shriveling or molding of caseless pupae and by the failure of cased individuals to emerge after 40d (these beetles typically emerge within 20d of the sealing of the pupal case; Brown and Funk 2005). There were no differences in initial mass among case (H_1 = 1.73, P = 0.19) or RH treatments (H_2 = 0.17, P = 0.92), or in pupal age among case (H_1 = 3.9, P = 0.052) or RH (H_2 = 3.80, P = 0.15) treatments.

To further evaluate the causes of emergence patterns via data not collected in the initial trials, we conducted a follow-up experiment in which 40 cased and 20 caseless pupae were maintained at medium RH for up to 32d. Cases that did not yield emergent adults were then opened. All cased and caseless test individuals could thereby be assessed for possible developmental abnormalities and, especially, the death of mature adults prior to emergence from the case.

Effects of RH on case strength

Pupal cases from which adults did not emerge in the survival experiment were subsequently redistributed across RH treatments and placed in the appropriate incubators for 23d. We then tested the effects of humidity conditions on case wall strength. Two different approaches were used, to evaluate the robustness of our results. First, a modest number of cases were tested using a “case cracker” similar to that used and diagrammed by Otto and Svensson (1980). This is a somewhat crude method because of its incremental loading of different masses and because the surface area to which pressure is applied, and thus force, cannot be readily quantified (Sanson 2006). Second, the absolute force needed to break the case wall was evaluated with spring-loaded fruit pressure testers (penetrometers) (McCormick Fruit Tech, Yakima, Washington), each fitted with a cylindrical plunger that was flat-tipped and thus of measurable surface area (as in Dumont 1999). To standardize the speed, angle, and location at which the case wall was contacted by the plunger, the penetrometer was mounted on a sleigh that was pulled along a metal track attached to a wooden base using a crank and pulley system to move it towards the case (Fig. 2). The case itself was glued at its base (Fig. 1b) to a piece of cardboard held in place by a wooden vice; it was further supported from behind by the vertical wooden wall of the device. The crank was then slowly turned until the wall of the case was broken by the penetrometer, which provided a reading of the pressure (in grams) necessary to do so.

To accommodate the range of observed pupal case strengths, we needed to use two different penetrometers, capable of measuring pressures of 0 - 500g and 0 - 13kg, respectively. To compare the results from these two devices most accurately, we scored half of the medium RH cases with one penetrometer and the rest with the other. We then standardized our results by multiplying each measurement from the larger penetrometer by the ratio of the two resulting means.

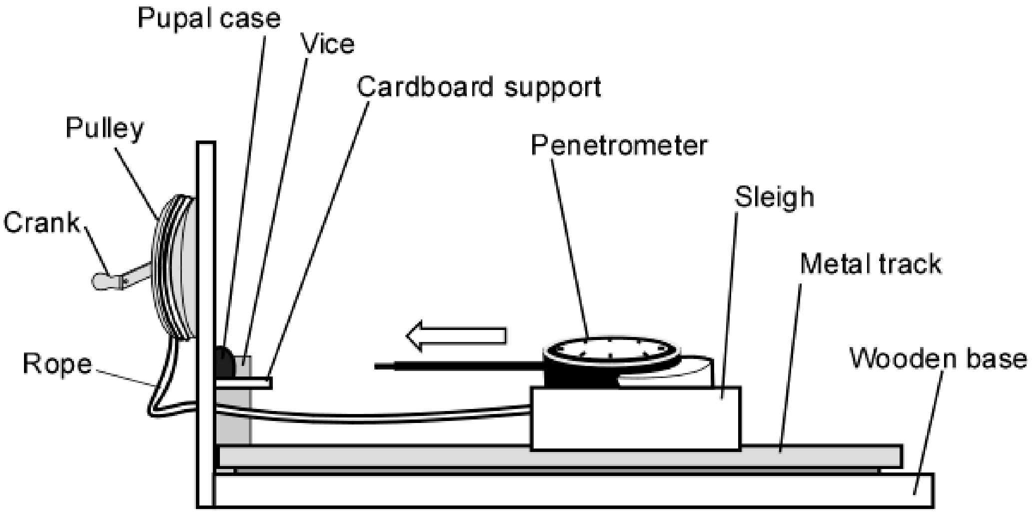

Figure 6-2. Diagram of the sleigh-based crank and pulley system used to standardize the manner in which the penetrometer contacted the case and measured its strength. See text for further details.

Effects of RH on predator handling time

Knowing that *Neochlamisus* fecal cases can reduce threats from predators (Brown and Funk in review), we evaluated whether humidity influenced case strength sufficiently to affect predation resistance. To do so, pupal cases from each RH treatment were individually glued (in its natural orientation) to a piece of paper and exposed to a generalist predator, the house cricket *Acheta domestica*. Each cricket was starved for 3d and then placed in a 9cm Petri dish arena for a 1.5h acclimation period prior to the placement of a single pupal case in the center of the arena. We then recorded the time required for a cricket to chew through the case wall following initial attack. Each such trial was observed for 2h or until the case was penetrated. If the cricket did not attack during this period, the case was later paired with another cricket. No cricket was used in more than one trial. 88% of trials yielded an attack.

Results

Effects of case on surviving submersion

Larvae expressed an ability to survive submersion for considerable periods of time (Fig. 3a). For example, approximately half of the larvae subjected to the 8-hr trial survived and some survived the 32-hr trial as well. There was no difference between the caseless and cased treatments in the maximum submersion length survived by individuals ($H_1 = 0.14$, P = 0.71). However, cased larvae were more likely to float (76%) than caseless larvae (49%) ($G_1 = 8.63$, P = 0.003). This pattern held for all scored trials, except the final one, which treated only four individuals (Fig. 3b).

Effects of case on pupal mass changes as a function of RH

Nineteen pupae died during the five-day trial and were excluded from analysis. Most (76%) of the surviving test animals had metamorphosed into adults during the trial period, but the likelihood of reaching this stage was even across treatments (G_2 = 5.33, P = 0.37). Low RH individuals weighed significantly, although not appreciably, less than high RH individuals (Fig. 4a). But no case effects were observed.

Effects of case on survival and adult emergence as a function of RH conditions

No difference was found in the percentage of caseless pupae surviving to maturity across RH treatments (G_2 = 1.16, P = 0.56). Cased and caseless pupae did not differ in survival under high RH (G_1 = 0.14, P = 0.71) (Fig. 4b) or in the time to reach maturation across RH treatments (H_2 = 3.34, P = 0.19). High RH cased beetles took significantly longer to be observed as adults than caseless ones (H_1 = 5.47, P = 0.02). However, the mean difference was about 2d, which is similar to the time newly eclosed adults remain inside their cases prior to emerging (Brown and Funk 2005). Thus, major patterns of development and survival were similar across these treatments. In striking contrast, *none* of the cased individuals maintained under low and medium RH emerged from their cases as adults (Fig. 4b), apparently dying instead. Similarly, in the follow-up experiment, none of the cased beetles emerged from their cases. When these cases were opened , we found that 88% contained fully developed, apparently normal, but dead, adults, a figure nearly identical to the 85% of caseless individuals yielding adults (G_1 = 0.07, P = 0.79).

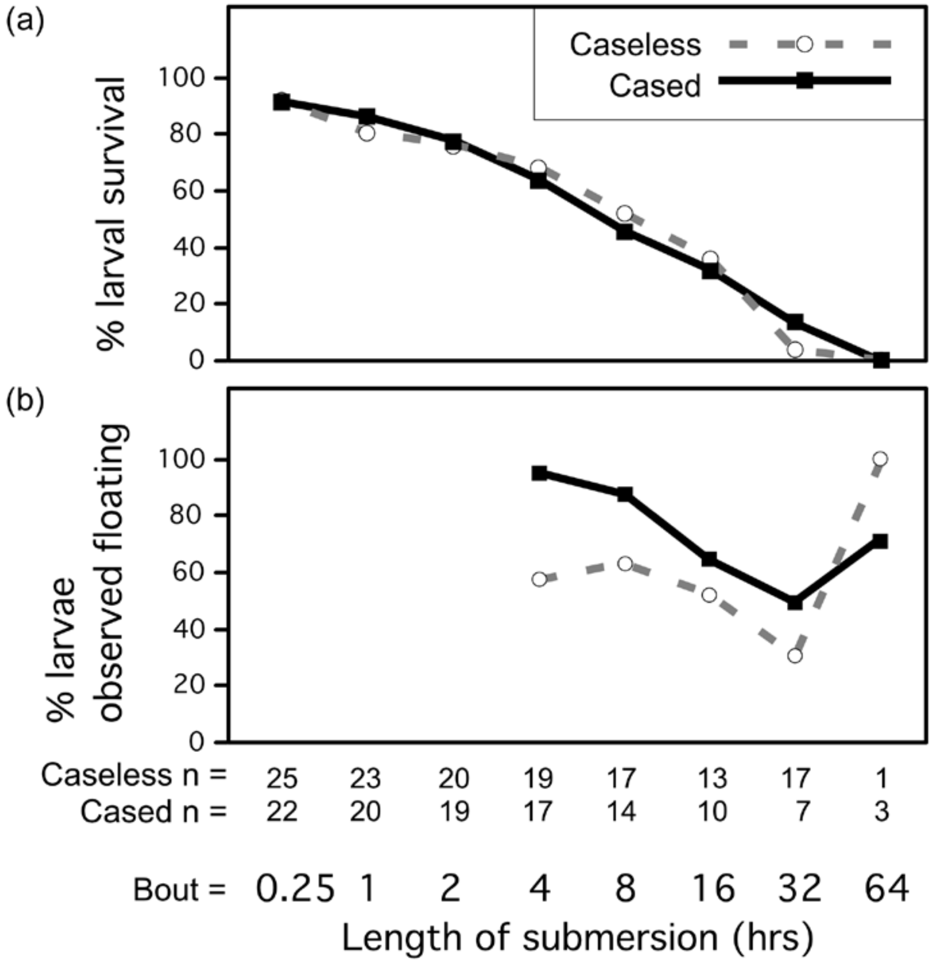

Figure 6-3. (a) Percent of larvae that survived after each trial of the submersion experiments. Surviving individuals were used in the subsequent trial. (b) Percent of larvae observed floating one hour into each trial of the submersion experiments for which observations were made.

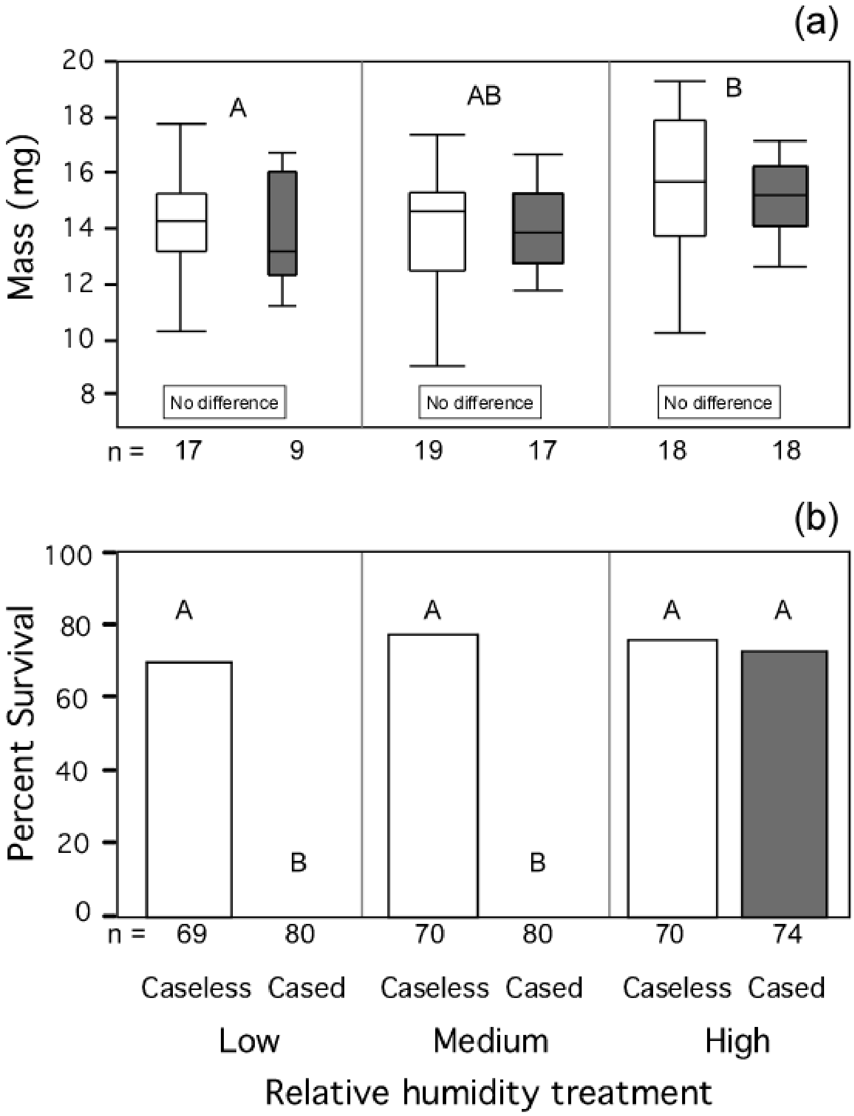

Figure 6-4. Effects of RH treatment on pupal mass and survival. (a) Mass of individual pupae after 5d. For "Cased" pupae, individuals were removed from cases prior to weighing. Box plots depict 10th, 25th, 75th, and 90th percentiles and median. (b) Percent of caseless pupae surviving to maturity and of cased pupae surviving until adult emergence from cases. Results from Kruskal-Wallis tests followed by Dunn's post-hoc test for differences among treatments.

Effects of RH on case strength

During the previous experiment, we noticed an apparent difference in the hardness of cases from the low/medium (= harder) versus the high RH treatments (= softer). Our experimental evaluation strongly supported this hypothesis. Cases maintained under low/medium RH required significantly and considerably greater pressures to break than those maintained under high RH using both the case cracker (Fig. 5a) and the penetrometers (Fig. 5b). By contrast, the low and medium RH treatments did not differ significantly in their resistance to penetration under either method.

Effects of RH on predator handling time

Crickets took significantly and considerably longer to chew through the walls of pupal cases maintained under low/medium as compared to high RH (Fig. 5c).

Discussion

It is tempting to assume the adaptive significance of conspicuous and presumably energetically costly building behaviors. However identifying and understanding specific functions requires empirical test and a consideration of how tradeoffs may influence these functions. As animal architectures are often made of pliant materials and are the product of behavior, they may be prone to phenotypic plasticity and thus to functional and fitness tradeoffs that vary across environmental contexts (e.g., Nislow and Molles 1993, Holomuzki and Biggs 2006). Here, we demonstrate such plastic ecological tradeoffs in the course of providing the first investigation how an abiotic factor (humidity/moisture)

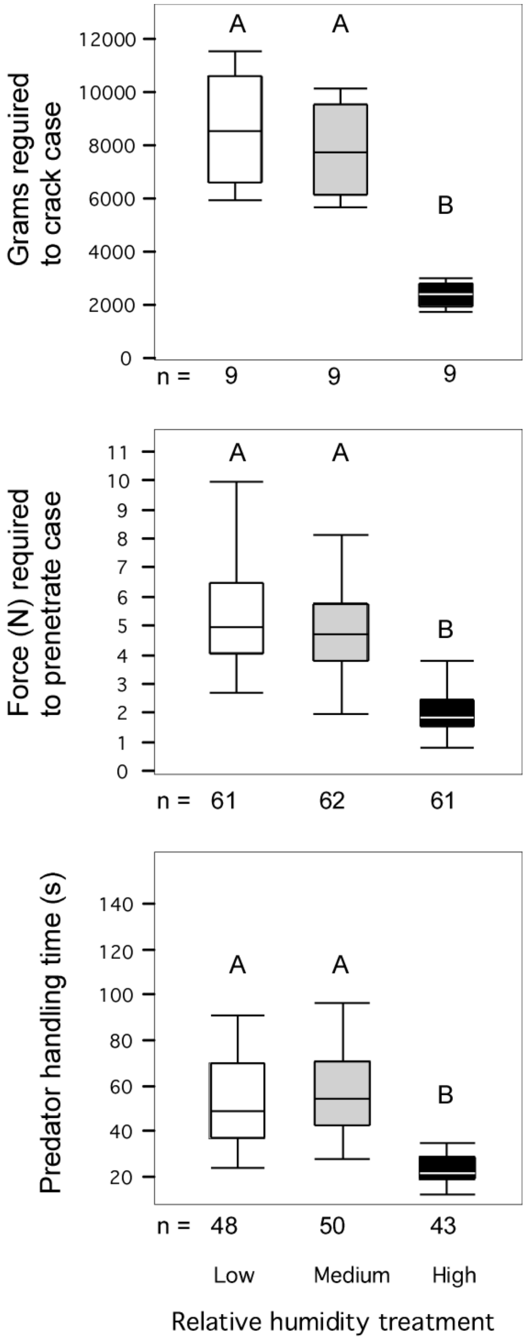

Figure 6-5. Effects of RH treatment on pupal case strength. (a) Mass required to break case wall using a case-cracker. (b) Force required to penetrate fecal case wall with a penetrometer. (c) Time required for house cricket to chew through case. Results from Kruskal-Wallis tests followed by Dunn's post-hoc test for differences among treatments. Box plots depict 10th, 25th, 75th, and 90th percentiles and median.

influences a remarkable example of animal architecture: the portable fecal cases *Neochlamisus* leaf beetles.

General Findings

Our findings did not support the hypothesis that cases protect larvae from drowning (e.g., by preserving pockets of air within them), an ecological plausibility given that larvae/cases are often covered with dew and that *Neochlamisus* species often live on plants that grow in, or along the edge of wetland areas. However, the extended survival of even caseless larvae while completely submerged and the greater tendency of cased larvae to float might indeed reflect adaptations to living in such habitats. We found no apparent affect of cases on desiccation or moisture absorption (as inferred from pupal mass) or on pupal maturation, although significantly elevated mass under high RH levels were observed. These observations do not support the hypothesis that fecal cases moderate humidity conditions. However, the ability of caseless pupae to survive at high rates under such low RH was surprising, especially given the reduced survival of caseless immatures reported elsewhere (Ellias and LeRoux 1964, Karren 1964, Erber 1988, Boldt and White 1992, Flinte and Macêdo 2004, Brown and Funk 2005). This could reflect specifically pupal adaptations to varying humidity conditions, reflecting the fact that pupae have no means of behaviorally adjusting their relations to moisture via drinking or moving their (fixed) cases. By contrast, larvae can do both and represent most of the observations cited above.

Intriguingly, while pupae appeared to reach adulthood with the same rate of success, whether cased or caseless, across RH treatments, those pupae maintained under low/medium RH completely failed to emerge from these cases, dying instead. Given that caseless pupae survived to maturity at high rates under low/medium RH it seems unlikely that increased adult susceptibility to desiccation explains these patterns. Having noticed that low/medium RH cases seemed harder/stronger than high RH cases, we therefore alternatively propose that adult mouthparts were simply not strong enough to cut free of the harder low/medium RH cases. The fact that these beetles were at least trying to do so is supported by a single individual that did successfully cut away at the case, yet still failed to escape it. Further evidence for this hypothesis was provided by our finding that low/medium RH pupal cases were indeed significantly and considerably stronger than those maintained under high RH. Thus, it seems that case strength responds plastically to environmental RH, with important fitness consequences (Fig. 6).

Further experiments demonstrated that RH-induced case strength also imparts important fitness variation via a different function and, critically, in the opposite direction. Specifically, the harder cases produced by low/medium RH yielded greater resistance to cricket predators than did the softer cases yielded under high RH (Fig. 6). By increasing predator handling time (and associated energetic costs), harder cases raise the cost:benefit ratio to predators while increasing opportunities for prey escape (Schoener 1971, Stephens and Krebs 1986). In sum, the phenotypically plastic response of cases to humidity variation imposes a substantial and environmentally moderated fitness tradeoff with respect to two key aspects of *Neochlamisus* ecology.

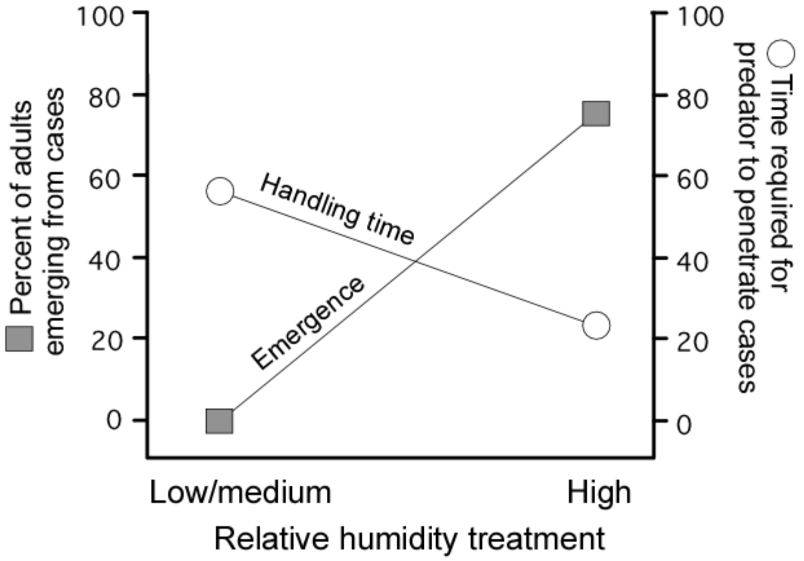

Figure 6-6. An ecological tradeoff reflecting the divergent effects of environmental variation on different aspects of fitness, as mediated by phenotypically plastic fecal cases. Under low/medium RH, the case becomes quite hard (Fig. 5), significantly increasing the (handling) time required for a predator to chew through it and gain access to the animal inside. However, adult beetles are apparently not strong enough to free themselves of such cases with their mandibles, leading to death (Fig. 4b). By contrast, for cases maintained under high RH, adults are highly capable of emerging, but the case is more quickly breached by predators.

Factors influencing case plasticity and tradeoffs

Such tradeoffs can play major roles in the evolution of phenotypes (DeWitt et al. 1998, Pigliucci 2001). Indeed, the nature of variation in the factors determining the relationship between humidity and the trait (case hardness) that mediates the tradeoff demonstrated here , will influence how and to what degree it effects the ecology and evolution of these insects and of this peculiar and exceptional example of animal architecture. Some of the potential implications of these factors for *Neochlamisus* immatures are explored below. Most of these issues are in need of rigorous evaluation.

Ecological - The tradeoffs of *Neochlamisus* cases, as influenced by the interplay of two ecological components (i.e., RH and predators), may be less dramatic if these conditions are correlated. For instance, exposed leaf surfaces may feature increased access by predators (as in Bernays 1997), but lower humidity due to sun and wind. Thus, cases might tend to be stronger when predators are present. By contrast a larva tucked into the deeper shaded and protected recesses of the host plant may experience higher RH (and thus softer cases) while being threatened by fewer cruising predators. Such tendencies would require case hardness to vary over short timescales. Such patterns have been commonly observed in the field as a consequence of diel variation in weather (e.g., rainy versus sunny) (DJF *personal observations*).

Interestingly, we found that RH as high as 75% was not sufficient for successful adult emergence, even though many *Neochlamisus* populations live in areas where the daily maximum RH would typically not exceed this value. One might thus conclude the persistence of these beetle populations in such localities to be paradoxical. However, ‘typical’ RH values can reflect appreciable local variation over modest temporal scales.

This, combined with the above observations on microhabitat differences and rapid changes in case hardness could account for this incongruity by providing immatures with opportunities to coordinate activities with these sources of variation. The timing of a plastic response is especially important when environmental conditions are unpredictable (Padilla and Adolph 1996). Thus, the particular speed with which case strength changes should greatly impact the biological implications of this ecologically dynamic tradeoff.

Behavioral - These tradeoffs may also be moderated through behavior, whose plasticity allows rapid responses to environmental variation (Sih 2004). For example, larvae often move within a host plant (*unpublished data*) and might thus influence case strength by feeding on different plant tissues or by regulating exposure to different humidity and moisture levels (e.g., dew, rainfall). Larvae could reserve case construction (which requires cutting through the case prior to addition of new material) and adult emergence for moist nights or dewy mornings and may increase case strength by exposing themselves more during sunny periods (DJF *personal observation*). Behavior could also be used to manipulate the predatory aspects of this tradeoff, for example, by frequenting sites of low predator density or by increasing case strength through the addition of materials such as trichomes, as in *N. platani* (Brown and Funk *in review*).

Larvae determine the sites where the immobile pupal cases will be fixed during metamorphosis and older larvae often move to new sites on their host plant or onto other plants prior to pupation (Brown 1943, Erber 1988, Neal 1989, Brown and Funk 2005, *unpublished data*). This behavior could reflect a search for pupation sites of appropriate RH, such as the fork of branching limbs where dew might collect (insuring easier adult escape from the case) or the tips of dead branches (increasing case hardness and thus protection from predators while immobile). Both these tendencies have been commonly observed in the field (DJF *personal observation*).

Genetic - Building behaviors themselves can have a strong genetic basis (e.g., Dawson et al. 1988), yet nothing of the genetics or heritability of case-building behaviors is known for any camptosomate. Since the environmental factors influencing *Neochlamisus* fitness (i.e., RH and presence of predators) may vary rather unpredictably, evolving genetically fixed rather than environmentally plastic means of responding to these factors might be expected to yield a less-than-optimal phenotype in most situations (e.g., Padilla and Adolph 1996, Sih 2004). However, Hoverman and Relyea (2007) found that when snails faced multiple types of predators, induced shell shape tended to respond to the most powerful selective pressure rather than to some combination of pressures. Therefore, if a camptosomate species experiences one selective pressure that is more powerful, or a selective scenario that is consistent and predictable, such as low humidity and few predators, then selection may be expected to promote the evolution of less plastic and more genetically-based case structure, for example, one that ensures the production of softer cases to maximize adult emergence (e.g., Price et al. 2003). Clearly the degree to which the presently documented ecological tradeoff in case-making behaviors is genetically hard-wired versus environmentally plastic should vary according to patterns of environmental variability in relevant parameters (for other ecological tradeoffs in architectural traits, see Nislow and Molles 1993, Zamora-Munoz and Svensson 1996, Wissinger et al. 2006, Holomuzki and Biggs 2006). Such ecological ’extrinsic’ tradeoffs thus provide an interesting contrast to the sorts of tradeoffs more commonly invoked for building behaviors, which involve ‘intrinsic’ factors such as energy and resource allocation (Hansell 2005), that tend to affect individuals in different ecological situations more similarly and thus do not provide variable pressures that result in architectural variation

Geographic/Temporal – Humidity-mediated case strength could be responsible for geographic variation in case design in relation to susceptibility to attackers and emergence times of adults (for the relationship between plasticity and geographic variation, see Miner et al. 2005, Fordyce 2006). Camptosomate taxa exhibit intriguing variation in elements of case shape and material (Erber 1988) and live under disparate ecological conditions. Consider two examples. First, the *velutinus* group of *Neochlamisus* resides in southwestern North America (Karren 1972) in habitats where summer RH is generally much lower than in the habitats of species studied here. Second, other camptosomate larvae are inquilines in ant nests and spend their entire juvenile lives underground (Erber 1988), where they constantly face attack from their ant hosts, while possibly experiencing consistently high humidity levels (Heller and Gordon 2006, Bollazzi and Roces 2007).

If the fecal cases of desert species respond to low humidity in the manner documented here, they must employ special strategies to escape from them as adults. Two South American camptosomates do so at the beginning of the rainy season (Flinte and Macêdo 2004, Reu and Del-Claro 2005) and desert species (or all *Neochlamisus* for that matter) could delay pupation or adult emergence until conditions allow appropriately softened case material. Alternatively, larvae might alter case composition as a function of local conditions, for example, by using more or less of the ‘cementing agent’ hypothesized to account for case robustness.

The eastern species of *Neochlamisus* alone includes several taxa with distributions that span considerable variation in latitude, altitude, and habitat. Perhaps the extreme example is offered by the red maple (*Acer rubrum*, Aceraceae) host form of *Neochlamisus bebbianae*, whose host plant is the most widely distributed North American tree and whose distribution parallels that of its host (DJF *personal observations, unpublished data*). Thus, these beetles are found from Florida to Quebec and from the Atlantic coast to the Midwest, in habitats ranging from northern bogs, to sandy-soiled pine barrens, to upland forests. This great variety of ecological situations should be expected to select for variable case characteristics and associated behaviors and would provide an ideal system for investigating the ecological factors driving the evolution of these traits. Likewise, the distribution of the host plant, and presumably the beetles, has been affected by Pleistocene glaciation (Martínez-Meyer and Peterson 2006). Thus, such temporal factors also need to be taken into consideration when evaluating these traits.

Conclusion

This study provides the first direct test of the potentially adaptive significance of fecal cases in relation to abiotic factors. Its most notable finding is a striking environmentally influenced ecological tradeoff that has great implications for *Neochlamisus* leaf beetle fitness and for the evolution of the unique animal architectures that are at the heart of this tradeoff. Specifically, we find that the pupal fecal cases of *Neochlamisus* become softer at high RH, allowing mature adults to readily cut free of the case and emerge from it, but also rendering them more susceptible to predation;

contrarily, at low humidities, the cases become too hard for adults to cut themselves free, yet yield greater resistance to predators. We consider the wide-ranging implications of this tradeoff, what its underlying basis may be, and how it may be regulated by the beetles to their advantage. These identify a diversity of important questions that should be the focus of future work that seeks to understand the mechanistic, behavioral, ecological, geographic, temporal and evolutionary dimensions of this tradeoff and this trait. To these we would add the value of comparative phylogenetic investigations aimed at tracing case evolution across the camptosomates and rigorously evaluating the association of particular case traits with particular ecological factors. To date, detailed phylogenetic analyses of animal architectures have been few, having been conducted most thoroughly in a few insect groups (e.g., Stuart and Currie 2001, Bucheli et al. 2002) and birds (Hansell 2000). In sum, we offer the diverse fecal cases of camptosomate leaf beetles as underexploited and potentially highly informative vehicles for studying the ecology of animal architecture.

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CHAPTER VII

THE ROLE OF FECAL CASES, PLANT HAIRS, AND MICROHABITAT SELECTION IN THE BEHAVIORAL THERMOREGULATION OF *NEOCHLAMISUS* LEAF BEETLES

Abstract

Nearly all insects can control their internal temperature to some degree, either autonomically or behaviorally, with methods like shivering, basking, or microhabitat selection. Many small animals build elaborate structures, such as nests, to modify abiotic conditions, including temperature. Here, we present the first tests of the thermoregulatory properties of one such animal architecture: the portable fecal cases of the leaf beetle group Camptosomata. We find that case-building and related behaviors may serve to prevent overheating. Using thermocouples inserted into pupal cases arranged naturally on a leaf in the field, we find that cases with embedded plant hairs maintain internal temperatures lower than ambient. In the laboratory, we found that cases slow acclimation to surrounding temperature when moving from cool to hot environments, but not vice versa. By observing larvae in the field, we find that the proportion of time spent on the cooler undersides of leaves is positively associated with ambient temperature, consistent with behavioral thermoregulation. As temperatures rise, larvae hold their cases above the substrate more frequently, likely facilitating cooling while also increasing vulnerability. Trade-offs between the anti-predatory and thermoregulatory functions of cases and larval behavior are discussed.

Introduction

The small size of most insects allows them to reach niches unavailable to larger organisms and they have successfully taken advantage of this fact. However, their higher surface area-to-volume ratio means that they will be more drastically affected by fluctuations in abiotic environmental factors such as temperature and humidity than larger animals (Casey 1981, Heinrich 1993). Body temperature on this scale, for instance, can change immensely in a short time due to slight changes in sun/shade and wind conditions (Casey 1981). Despite this, some insects are able to keep their body temperature (T_b) well away from ambient air temperature (T_a), or within a quite specific range of temperatures (e.g., Casey 1976). They do so through various methods of thermoregulation (Casey 1981, Heinrich 1981, 1993, 1996).

Large animals may regulate their internal temperature entirely through physiological means. Some large insects maintain T_b above T_a by producing heat internally (endotherms), usually by vibrating the flight muscles (Heinrich 1974). However, small insects typically cannot produce enough heat to compensate for that lost through the cuticle (Casey 1981) and most are just assumed to be thermal conformers (ectotherms) (Kammer 1981, Heinrich 1993). Even so, growth rate and performance of ectotherms are often temperature-dependent (Huey and Hertz 1984, Kingsolver and Huey 2008) and while fewer studies investigate the thermoregulatory capabilities of really small organisms, such as insect larvae, they too can control their temperatures through behavioral means (Casey 1981), such as basking (Heath 1967, Casey 1976), aggregating (Seymour 1974, Grodzicki and Caputa 2005), and microhabitat selection (Ruf and Fielder

2002, Hausmann et al. 2005, Kührt et al. 2005). Indeed, many insect larvae are thus active over a wide range of temperatures (Kammer 1981, Heinrich 1993).

Instead of changing their internal temperature directly, some small animals actually alter their surrounding environment to better suit their needs. By building shelters, numerous animal taxa protect themselves from less than ideal biotic and abiotic situations. Animal architectures alter temperature conditions by increasing heat conduction, absorbing solar radiation, and providing shade (Grigg 1973, Joos et al. 1988, Ruf and Fielder 2002, Edgerly et al 2005, Danks 2002, Hansell 2005). Building behaviors can aid evaporative cooling (Seymour 1974, Korb 2003) and, alternatively, insulate against normal levels of heat loss felt by small organisms (Bult and Lynch 1997, Hieber 1985, Rivers et al. 2002). Other architectures help builders survive extreme temperatures such as sub-freezing (Sakagami et al. 1985, Rivers et al. 2005) and high heat (Knisley and Pearson 1981, Schuurman and Dangerfield 1996). Unlike other forms of thermoregulation, animal-built structures most often aid flightless immatures and immobile stages, such as eggs and pupae, which cannot orientate themselves toward optimal temperatures and thus are especially vulnerable to fluctuating external conditions (Heinrich 1993, Danks 2002, 2004). Constructions also increase the ambient temperatures in which a builder can be active, increasing growth rate and vigilance (Hansell 2005).

Although unusual by human standards, fecal architecture can also aid thermoregulation. However empirical studies are few and none are mentioned in a recent review of the ecological impacts of insect feces (Weiss 2006). Seymour (1974) discovered that *Perga dorsalis* sawfly larvae react to high temperatures by excreting a watery pellet from the anus and applying it to the ventral side of the abdomen. Individuals within an aggregation share the fluid between themselves until they are all quite wet. This behavior lowers individual T_b, possibly by increasing evaporative cooling. Nearly 20% of leaf beetle species (Chrysomelidae) are known to cover their eggs and/or larval abdomens with feces in some form (Vencl et al. 1999). Some species cover their dorsa in loose, wet feces, while others build more permanent defenses. It has been suggested that this fecal retention can be thermoregulatory as well as a defense from predators (Olmstead 1994, Eisner and Eisner 2000, Schaffner and Müller 2001, Chaboo 2007, Chaboo et al. 2008). Tortoise beetles (Cassidini) create a "shield" by holding a loosely attached platform of their own waste material over their backs. Heinrich (1993) observed that tortoise beetle larvae orient their shields in response to solar radiation, much as they do toward potential predators, and suggested this behavior was an attempt at thermoregulation. In addition, Bacher and Luder (2005) found no effect of fecal shields toward lowering the fatal effects of UV-B radiation, but beyond these introductory observations, no one has tested if fecal structures of the chrysomelids alter larval temperature.

Perhaps the most elaborate fecal structure is found in the leaf beetle group known as Camptosomata. These casebearers create a "case" of fecal material around their eggs and which larvae maintain throughout the juvenile stadia (Erber 1988). The larval case contains an opening at one end for the larva's head and legs, but the case is sealed to the substrate for pupation. Once an adult, the beetle cuts a circle around the apex of the case and crawls out. See Brown and Funk (2005) for more details of case-associated life history. Since camptosomates spend so much time nearly completely surrounded by the walls of their cases, these fecal structures are more likely to affect thermoregulation than the shields of the Cassidinae. In fact, larval casebearers have a less sclerotized cuticle and perhaps simpler spiracles than related larvae (LeSage 1982, Root and Messina 1983), which may have affected their heat exchange and reduced their ability to maintain thermal homeostasis without a case. Fecal cases are often much darker than the larval body, thus potentially altering radiative absorption, which, along with conductive cooling, typically impose the greatest impact on an insect's internal temperature (Casey 1981, Danks 2004). We hypothesize that any object enclosed in a fecal case, especially a sealed pupal case, experiences different internal mean, maximum, range, and time to acclimation to environmental conditions than one without this barrier. Additional architectural components are expected to further affect the thermal properties of fecal cases. For example, *Neochlamisus platani* incorporate hairs from their host plant (i.e., trichomes) into and onto the case wall, often masking the fecal material of the case. Since these plant hairs add another layer to the fecal material and alter case color, we hypothesize that they also alter internal case temperature.

In this study, we present the first empirical tests examining how fecal cases affect internal temperatures, using two closely related *Neochlamisus* spp. *Neochlamisus* are herbivores that feed on exposed leaf surfaces, which gather large amounts of solar radiation, and so their immatures are likely to be affected by the thermal properties of fecal cases. The genus *Neochlamisus* also contains variation in case construction, such as trichome use, which may further affect temperature. Here, we explore the relationship between location on the leaf surface and the presence/absence of cases and trichomes on temperature under field conditions. We then test the ability of cases to slow the time it takes to reach surrounding temperatures under laboratory settings. In addition, since moving within the habitat is the most common and effective way insects thermoregulate (Casey 1981) and we are interested in the thermoregulatory behaviors of this group, we investigate the responses (such as microhabitat selection) of active larvae to natural temperature changes in a field setting. Fecal cases are a marvel of the natural world, yet very little is known about their potential affects on the life history, development, and evolution of these amazing animal architects.

Methods

Study organisms

For this study, we used pupal cases and larvae of *N. platani*, which lives on American sycamore, *Platanus occidentalis*, and empty pupal cases of *N. bebbianae* from populations that feed predominantly on red maple, *Acer rubrum*. *N. platani* were collected as laboratory-laid eggs from adults collected in and around Nashville, TN in the spring of 2007. *N. bebbianae* were collected from their host plant as larvae in Vermont during the summer of 2007. Larvae were reared individually on their respective host plants in 5cm Petri dishes with moistened filter paper at 24 C and 14:10 light:dark cycle until use or pupation. Pupal cases were stored in a -80 freezer until use.

Effects of pupal cases, trichomes, and leaf position in the field

To produce pupal cases "with trichomes" and "without trichomes," *N. platani* larvae were reared on manipulated leaf clippings from sycamore trees. Leaves were collected in Nashville and transported to Vanderbilt in an iced cooler. In the lab, the leaves, which are normally densely covered in trichomes, had one side denuded of trichomes by rubbing gently with a gloved finger, while the other side was left pubescent. Each side was then cut into pieces and distributed to the respective larval treatment. This method of trichome removal does not seem to affect the surface cells on the leaf and has no effect on larval survival to pupation (Chapter V). The day before testing, a 30-gauge Type-T thermocouple was inserted into the ventral seam of each pupal case. The entire soldered surface of the thermocouple was surrounded by case material, so that only temperatures inside cases would be measured. The wire attachment was then firmly attached and any gaps sealed with wood glue, taking care to minimize coverage on the outside of the case. Each thermocouple was attached to a single-channel datalogger (LogIt LTC, Contoocook, NH) set to measure temperature at ten-minute intervals.

The next day, six thermocouples were attached to a single, horizontal leaf (40-80 cm above ground level) of a potted sapling sycamore located in a field on a forest edge in Nashville, TN, simulating the natural position of sycamore trees. Each side of the leaf (abaxial = bottom, adaxial = top) contained a thermocouple inside a fecal case with trichomes, a thermocouple inside a case without trichomes, and a thermocouple without any covering (caseless), creating six treatments per leaf (Fig. 1). Thermocouples were placed on leaves within 2 cm from the next thermocouple on the same leaf surface and, when possible, directly below/above a thermocouple on the other side of the leaf. Cases were attached to the leaf with wood glue in a similar orientation to natural pupation. Wires were attached with wood glue only on the coated portion of the wire (not affecting temperature readings). All wires (cased and caseless) were bent and attached so that the

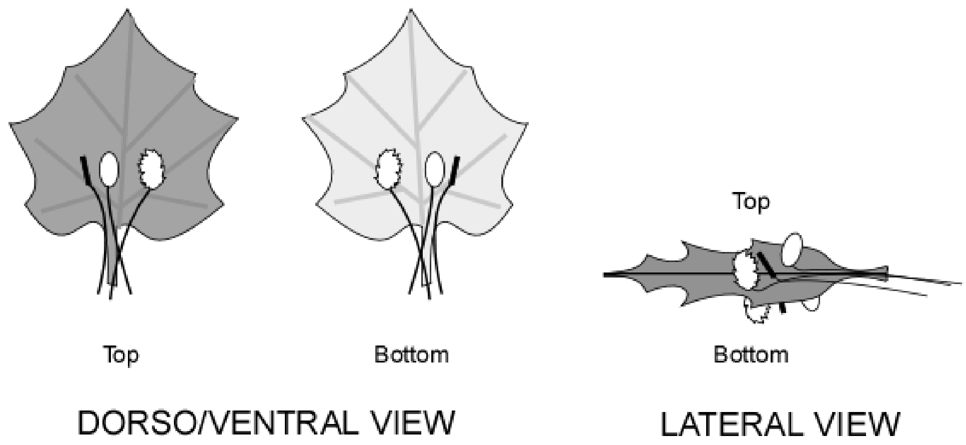

Figure 7-1. Diagrammatic representation of the six thermocouple treatments: uncovered thermocouple (caseless), thermocouple inside a pupal case with no trichomes, and thermocouple in case with trichomes on both the top and bottom of the leaf. soldered portion of each was perpendicular to the leaf surface and the tip was <5 mm above/below the leaf surface, but not touching it. Treatments remained in the field for at least one sunrise-sunrise period (or nycthemeron). Due to a limited number of available thermocouples, only one set of six treatments could be tested at a given time (= one trial) and not all treatments could be tested each trial. We performed seven trials, but one trial was removed from analysis due to a heavy rain that dislodged most of the thermocouples. Trees were unique to each trial.

Effect of pupal cases on time to reach equilibrium temperature

To test if fecal cases affect the time required to acclimate to environmental temperatures, empty *N. bebbianae* pupal cases implanted with a thermocouple (as above) and a thermocouple with no case (caseless) were simultaneously shifted between two incubators representing extreme natural conditions, one set to 4 C and the other 40 C. Four pupal cases were used, each experiencing one shift from 4 to 40 C and one shift from 40 to 4 C (= 4 trials). The direction of first shift was systematically varied between pupal cases. Only two thermocouples were used, but which thermocouple was inserted into a case and which was not was alternated between each trial. Thermocouples remained in each temperature environment for one hour before being shifted to the alternate incubator. Dataloggers recorded temperatures every five seconds. Data were analyzed as the time in minutes required to reach the temperature of a given incubator starting from insertion into that incubator.

Microhabitat selection of N. platani *larvae*

To determine if *Neochlamisus* larvae thermoregulate behaviorally via microhabitat selection or other behaviors, we observed 13 *N. platani* larvae (third and fourth instars) on three potted sapling sycamores in natural conditions. All larvae were individually marked with a small dot of model airplane paint (Testor's, Rockford, IL) and placed on the top surface of leaves the night prior to observations (19:30, with air temperature ~23 C). Such marking has been used in numerous experiments with no affect on larval behaviors (e.g., Brown and Funk 2005). Larval position, activity, and various temperatures were recorded every 20 min from just before sunrise (06:40) to just after the sun set behind the treeline (19:00) on September 16th, 2007. The following temperature readings were taken: 1) the external surface of each larva's case, 2) the adjacent leaf surface (mean from two measurements taken in different locations), and 3) the opposite side of the leaf. In order to take multiple measurements of an individual without disturbing its normal activity or case physiology, we used a handheld infrared thermometer (Omega, Stamford, CT) with an accuracy of ± 1.0 C and a 12:1 distance:dot ratio; thus we were able to measure the mean temperature of a ~1.25 cm diameter circular area, inclusive, when holding the device ~15 cm away. These measurements include the case's temperature and the temperature of its immediate surroundings.

Statistical analysis

Due to small sample sizes, an incomplete random block design, and large differences between trials (Fig. 2A), thermocouple data are analyzed as mean deviations from each trial mean, thus accounting for the effect of the trials, which would otherwise be treated as blocks. Data were analyzed with Kruskal-Wallis tests followed by Dunn's means comparison tests to inspect treatment differences (Dunn 1964, Zar 1996). We used Wilcoxon's signed-ranks test for two groups arranged as paired observations to determine significant differences between temperatures in the field observations. Proportions were analyzed using goodness of fit tests and G test of independence. Means are presented ± standard errors.

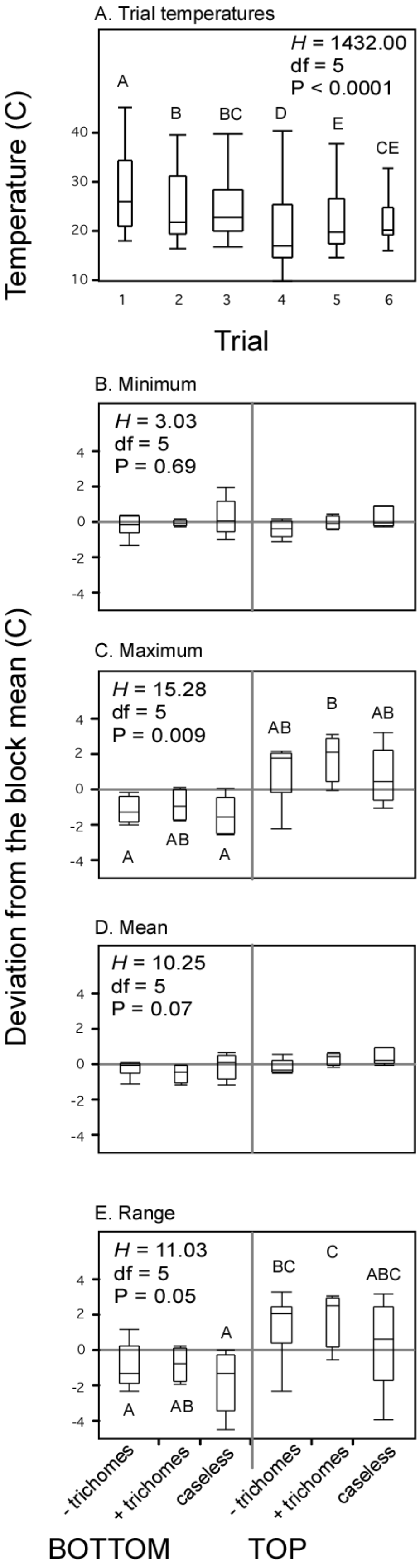

Figure 7-2. Effects of six treatments on temperature when taken every ten minutes in field conditions. (A) Mean temperatures of each trial +/- s.e. (day). B-E Mean deviations from the block (= trial) mean. (B) Minimum, (C) maximum, (D) mean, and (E) range of temperatures by caseless/cased, with trichomes/without trichomes, and on the top/bottom of leaves. Significance determined by Kruskal-Wallis tests followed by Dunn's means comparison tests to inspect difference among treatments. Box plots depict the 10th, 25th, 75th, and 90th percentiles and medians.

Results

Effect of pupal cases, trichomes, and leaf position in the field

Measurements began on the tail end of a record heat wave after which temperatures dropped dramatically, thus causing large differences between trials (Fig. 2A). We found significant differences in the maximum temperature reached and range of temperatures among treatments (Fig. 2C, E). To more clearly highlight the effects of the experimental categories, we then pooled case, trichome, and leaf location treatments with no significant differences (Table 1). There were no effects of cases or trichomes on temperature. Location on the leaf had the most affect on thermocouple temperature, with those on the top sides of leaves experiencing higher maximum, mean, and range.

To determine if cases or cases with trichomes alter the internal temperature from ambient over a range of temperatures, we separately compared hourly means from each treatment with and without trichomes to the adjacent (same side of leaf) caseless thermocouple (which recorded nearby ambient temperature). Figure 3 shows no effect of cases alone, but that cases with trichomes maintain an internal temperature less than ambient when ambient rises above ~20 C. Slopes of regressions between treatments with and without trichomes are significantly different (ANOVA, F = 47.62, df = 1, P <0.0001), and only that of cases with trichomes is different from $T_a = T_b$ (with trichomes: ANOVA, F = 47.62, df = 1, P < 0.0001; without trichomes: F = 0.24, df = 1, P = 0.63).

Table 7-1. Results of thermocouples inserted into various treatments in the field. Treatments with no differences (see Fig. 2) are pooled to compare the effects of cases, trichomes, and location on the leaf separately. Data are analyzed using Kruskal-Wallis tests on mean deviations from block means (*H*).

		Mean	S.E.	*H*	df	P	Interpretation
Minimum	Cased	-0.21	0.10	1.70	1	0.19	
	Caseless	0.38	0.38				
	- Trichomes	-0.31	0.15	0.93	1	0.33	
	+ Trichomes	-0.06	0.10				
	Top of leaf	0.02	0.24	0.38	1	0.53	
	Bottom of leaf	-0.02	0.19				
Maximum	Cased	0.14	0.38				Cannot be pooled due to differences in treatments. See Fig.1C.
	Caseless	-0.26	0.53				
	- Trichomes	-0.07	0.48	1.17	1	0.28	
	+ Trichomes	0.47	0.63				
	Top of leaf	1.11	0.27	13.81	1	0.0002	Top of leaf experiences significantly warmer maximum
	Bottom of leaf	-1.20	0.23				
Mean	Cased	-0.15	0.11	2.87	1	0.09	Cases experienced marginally significant difference in mean
	Caseless	0.27	0.29				
	- Trichomes	-0.19	0.12	0.21	1	0.64	
	+ Trichomes	-0.08	0.22				
	Top of leaf	0.26	0.19	3.75	1	0.05	Top of leaf experiences significantly warmer mean
	Bottom of leaf	-0.27	0.14				
Range	Cased	0.35	0.43				Cannot be pooled due to differences in treatments. See Fig.1E.
	Caseless	-0.64	0.71				
	- Trichomes	0.24	0.58				Cannot be pooled due to differences in treatments. See Fig.1E.
	+ Trichomes	0.53	0.67				
	Top of leaf	1.10	0.52	9.51	1	0.002	Top of leaf experiences significantly higher range
	Bottom of leaf	-1.18	0.35				

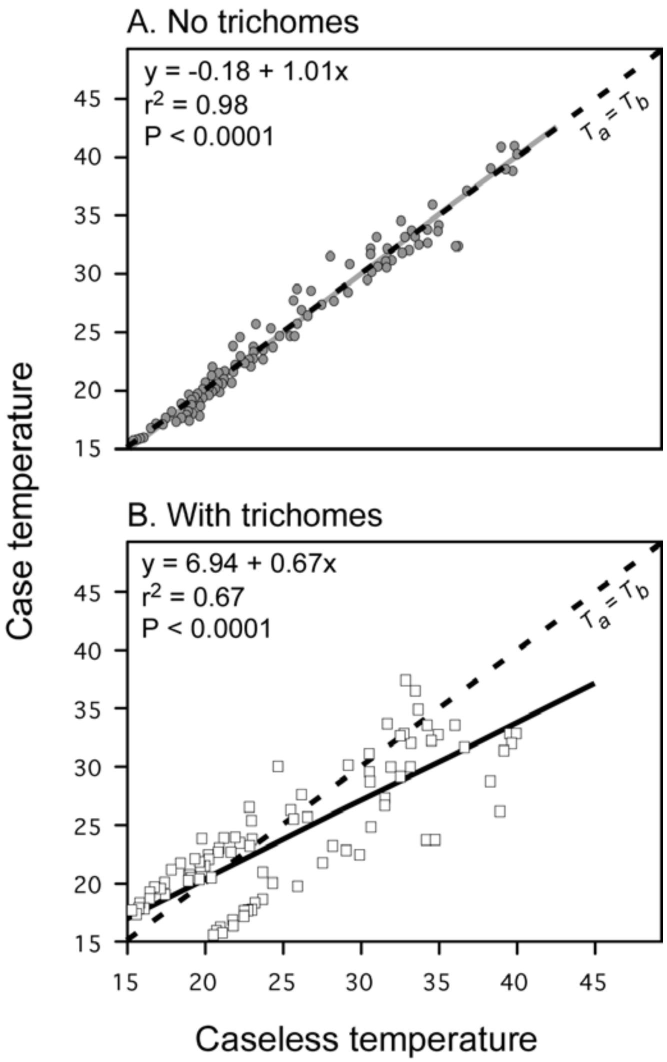

Figure 7-3. Effect of fecal cases on internal temperatures (hourly average of ten-minute measurements). Measurements within fecal cases (i.e., internal case temperature) are compared to ambient (the adjacent caseless thermocouple). Dotted black lines represent what would happen if there was no difference between temperature inside fecal cases and ambient. (A) Effect of fecal cases without trichomes (solid grey line). (B) Effect of fecal cases with trichomes (solid black line). Slopes of regressions between treatments with and without trichomes are significantly different (ANOVA, *F* = 47.62, df = 1, P <0.0001), and only that of cases with trichomes is different from $T_a = T_b$ (with trichomes: ANOVA, *F* = 47.62, df = 1, P < 0.0001; without trichomes: *F* = 0.24, df = 1, P = 0.63).

Effect of pupal cases on time to reach equilibrium temperature

Fecal cases greatly slowed the ascent in temperature from 4 to 40 degrees compared to thermocouples without cases (Kruskal-Wallis test, $H = 6.82$, df = 1, P = 0.009), but there was no discernable effect of cases when moving from an area of high temperature into one of low temperature (Kruskal-Wallis test, $H = 1.33$, df = 1, P = 0.25) (Fig. 4). We found no effect of the two dataloggers used (Kruskal-Wallis test, $H = 0.001$, df = 1, P = 0.96).

Microhabitat selection of N. platani *larvae*

Larvae were on stems and petioles only minimally (9% of observations) and were usually not settled during this time. Therefore only data from observations on leaves were analyzed. Larvae were on the bottom of leaves in significantly more observations than the top of leaves (Goodness of fit test, $G = 41.73$, df = 1, P < 0.0001). Individuals moved to the bottom during the morning and proportions on each leaf surface remained relatively steady throughout the rest of the day (Fig. 5A). Surprisingly, a few individuals moved to the top of leaves during the hottest part of the day (13:30-15:40), but the overall proportion on the bottom of leaves never fell below 55% after 08:00 (Fig. 5A).

Mean case temperatures did not significantly differ between leaf sides (top: n = 141, 18.90 ± 0.59C; bottom: n = 271, 19.41 ± 0.33C; Kruskal-Wallis test, $H = 1.14$, df = 1, P = 0.29). However, case temperature in direct sunlight (n = 86, 21.49 ± 0.73) was much greater than when shaded by clouds and foliage (n = 326, 18.64 ± 0.31) ($F = 16.08$, df = 1, P < 0.001), despite being an ephemeral condition. Perhaps intuitively, the likelihood that an individual was in direct sunlight was greater on the top of leaves (50%)

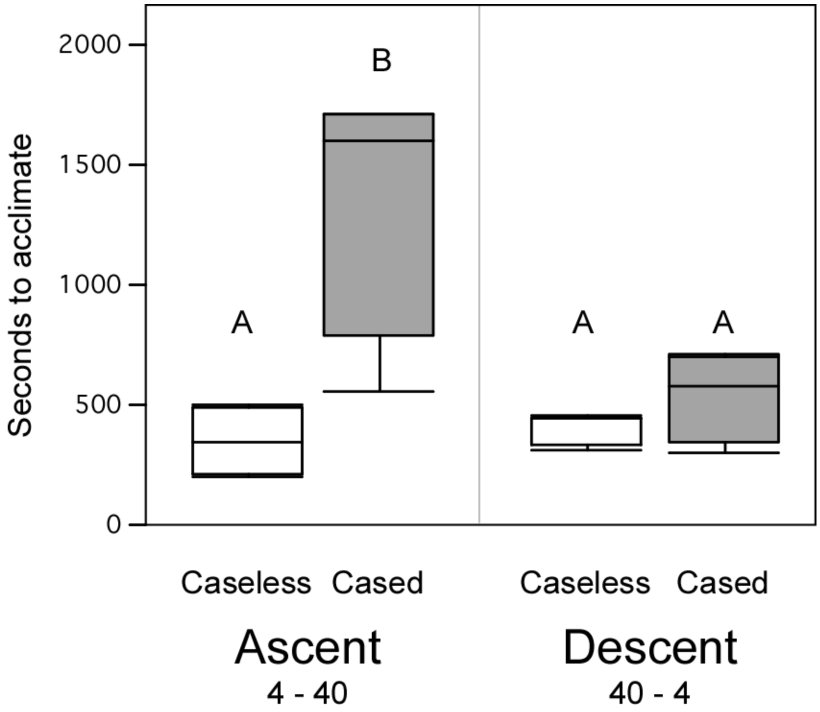

Figure 7-4. Time taken to reach a given temperature as measured by two thermocouples, one inserted into an *N. bebbianae* pupal case (cased) and one left exposed directly to ambient temperatures (caseless), when they are shifted between two incubators, one set to 4 C and the other 40 C. Box plots depict the 10th, 25th, 75th, and 90th percentiles and medians.

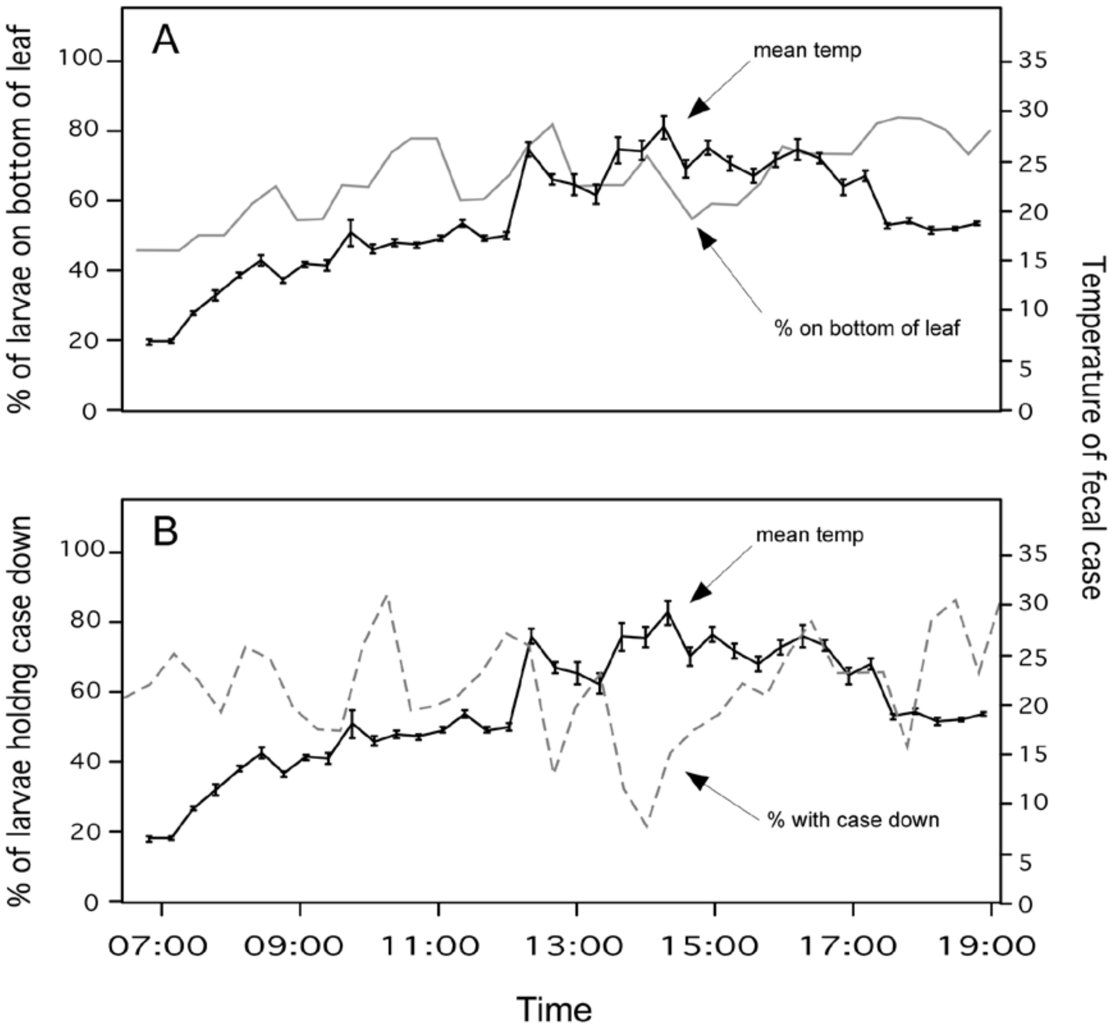

Figure 7-5. Average temperature of fecal cases of live larvae through time (black lines) compared to (A) the percent of larvae on the bottom of leaves out of total larvae observed on leaves (solid gray line) and (B) percent of larvae holding the case down tight against the substrate out of the total larvae observed that were either holding the case down or up above the substrate (broken gray line).

than the bottom (10%) (G test of independence, *G* = 84.48, df = 1, $P < 0.0001$). Cases experienced much higher variance in temperatures on the top of leaves than on the underside (Levene's test, *F* = 14.93, df = 1,410, P = 0.0001). In addition, when on top of leaves, larval cases were warmer than the opposite leaf surface, and when on the bottom of leaves, cases were significantly cooler than the opposite leaf surface (Fig. 6A). We found no differences between case and adjacent leaf surface temperatures when on either side of the leaf (Fig. 6A). When they were on the bottom of leaves, the larvae experienced a higher range of temperatures than did the bottom leaf surface, but not the top leaf surface. When on the top of leaves, however, larvae experienced a higher range than did the bottom leaf surface, but not the top leaf surface (Fig. 6B).

Larvae regularly exhibited four distinguishable actions: walking, feeding, resting with the case up (so that the legs are clearly visible), or resting with the case held down tight against the substrate (Fig. 7). Feeding and case building while cases were held down could not be distinguished. Larvae remained active over a wide range of temperatures (walking: 10.6-27.2C, feeding: 8.4-37.6C) and the mean temperature at which larvae performed each behavior did not differ (Kruskal-Wallis test, *H* =, df = 3, P = 0.60). Larvae were seen feeding more often on the bottom of leaves (n = 30) than the tops (n = 8) (G test of independence, *G* = 13.57, df = 1, P = 0.0002), consistent with observations of larval location in the field (Brown and Funk 2005). Larvae also spent proportionally more time resting with the case up on the top of leaves and more time with the case held down when on the bottom of leaves (G test of independence, *G* = 16.06, df = 1, $P < 0.0001$) (Fig. 5B). When on the lower leaf surface, larvae rested with the case raised when at a higher case temperature (n = 66, 19.13 ± 0.43C) than they rested with the case

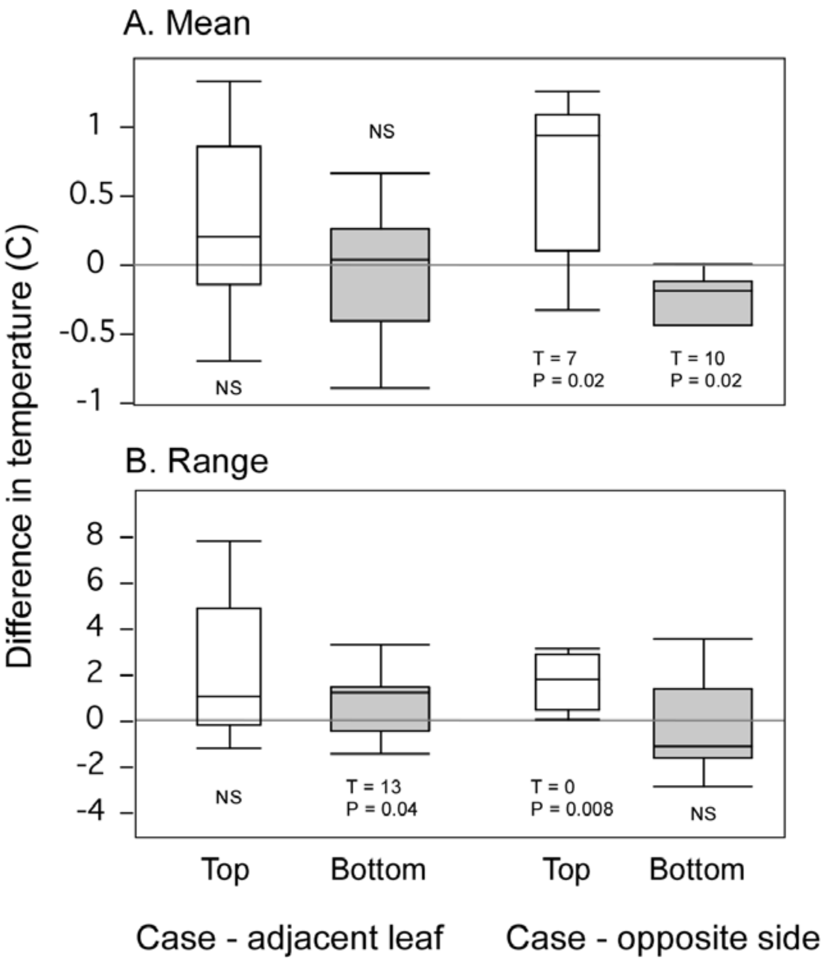

Figure 7-6. Differences in larval temperature compared to the adjacent leaf surface and the opposite leaf surface depending on which side of the leaf the larvae were on at the time (top or bottom). (A) Difference in means. (B) Difference in ranges. Tests are paired t-tests with a null that the differences = 0. NS = difference is not significant at P = 0.05. Significance was determined using Wilcoxon's signed-ranks test for two groups arranged as paired observations (T). Box plots depict the 10th, 25th, 75th, and 90th percentiles and medians.

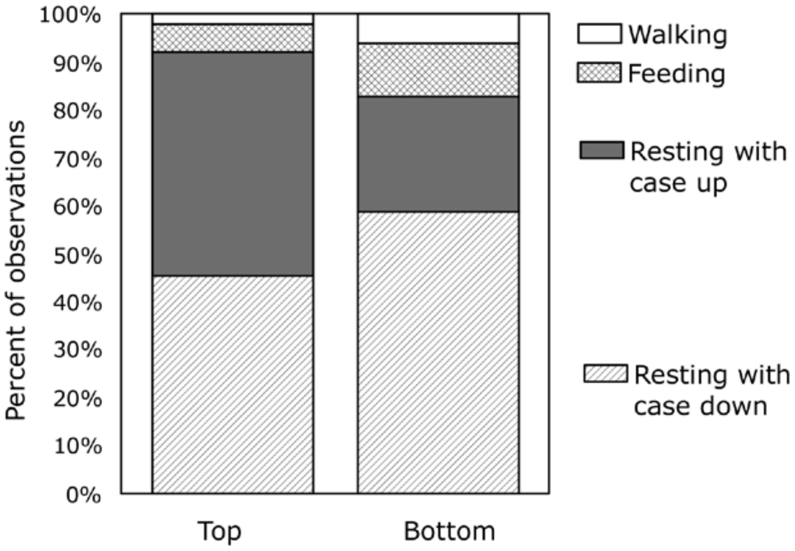

Figure 7-7. The proportion of observations in which larvae undertook a given action throughout the day according to location within the plant. Proportions are significantly different between locations (G test of independence, *G* = 23.40, df = 1, P < 0.0001).

held down (n = 159, 20.74 ± 0.65C) (Kruskal-Wallis test, H = 4.21, df = 1, P = 0.04). We found no similar effect of temperature on behavior when on the top of leaves (case up = 18.57 ± 0.92, case down = 18.6 ± 0.78, Kruskal-Wallis test, H = 0.04, df = 1, P = 0.84).

Discussion

General findings

This study provides the first empirical tests of the hypothesis that fecal cases and larval behaviors function to alter temperatures experienced by immature camptosomates. Temperature is often important for larval development and activity (Kingsolver and Huey 2008) and we expected cases and larval behaviors to control temperatures to which larvae are exposed. The *Neochlamisus* larvae studied here did not seek out overly warm or constant conditions and pupal cases did not increase the mean or maximum or reduce the range experienced. Instead, larvae were observed walking and feeding in a wide range of temperatures, including very low temperatures. Perhaps fecal cases increase the range of temperatures at which larvae can be active, although we expected this to be evident as a decrease in range inside cases (therefore maintain relatively constant T_b). There were, however, four lines of evidence to suggest that larval behavior and cases help prevent overheating.

First, *N. platani* cases with trichomes maintained temperatures lower than ambient when the environmental temperature rose above ~20 C. Structures like hair are effective at insulating, and thus reducing heat transfer in animals, even at the scale of insects (Kammer 1981). Kingsolver and Watt (1983) found that hair-like scales in *Colias* butterflies can prevent overheating during flight. More specifically, *Platanus* trichomes have been found to reflect radiation (Ntefidou and Manetas 1996). Therefore, trichomes embedded into fecal cases might similarly reduce overheating potential by insulating against heat gain.

Second and most impressive, fecal cases significantly increased the amount of time taken to reach 40 C when starting at 4 C, while there was no difference between the caseless and cased treatments on time to reach ambient temperature when shifted from 40 to 4 C. Increasing the acclimation period in this way could increase survival by slowing the rise in internal temperature until ambient temperatures fall again, allowing larvae and pupae to survive periods of otherwise detrimentally high ambient temperature. It would also provide larvae additional time to move to areas within the host plant that are cooler, such as in shade or the bottom of leaves, or to engage in activities that are limited to high temperature microhabitats. Slowing acclimation time may be most important for the immobile stages, such as eggs and pupae, which are completely sealed inside, and during which the insects cannot thermoregulate via microhabitat positioning and orientation.

The third was that temperatures on the bottom of leaves (which were more likely to be shaded from solar radiation) were significantly cooler than the top of leaves, and this is where larvae spent most of their time. Of course, there are many potential reasons why larvae prefer the bottom of leaves, such as reduced predation risk and a higher nutritional value of the leaf surface, yet there are also reasons why they might not prefer this side of the leaf. The undersides of *P. occidentalis* leaves are heavily covered in trichomes, which in many plants deter herbivores by blocking and entangling them (Levin 1973). Sycamore trichomes also slow down the movement of young *N. platani*

(Chapter V). In addition, unlike many other insects, young *N. platani* are capable of feeding on tough leaves as well as softer leaves (Chapter V) and therefore may be able to derive sufficient nutrition from the tougher leaf top. Regardless of the reason, larvae prefer the undersides of leaves, where they experience cooler temperatures than they would on the top of leaves.

Fourth, larvae tended to lift the case up over the substrate when their case temperature was high. Larvae were more likely to hold the case up during the hottest part of the day and when on the top of leaves, where they are exposed to higher solar radiation. We propose that holding the case up is a position taken when the temperature inside the case becomes too high and is therefore a form of behavioral thermoregulation. Holding the case above the leaf surface in this way might allow better air circulation or lift the insect above the leaf's boundary layer where radiative cooling is limited (Schuepp 1993). This behavior may be a last resort assumed only after the buffering effect of the case fails.

Fecal case evolution and temperature control

Camptosomates appear to have originated from areas with warm climates (Karren 1972) and may have evolved behaviors, including case building, to maintain relatively low T_b. Several *Neochlamisus* species are distributed throughout southwestern North America and probably evolved from more tropical species. The eastern North American species undergo most larval stages during the warmest part of the year (Brown and Funk 2005). Regardless of climate, even maintaining T_b below optimal temperature is often adaptive since fitness above optimal is worse than fitness below optimal temperatures

(Martin and Huey 2008). Therefore, casebearers could have faced risk of overheating during much of their evolution and any traits, such as those listed above, that lower this risk would be advantageous and maintained. Of course only two of our four lines of evidence suggest that fecal cases themselves might play a role in preventing overheating, meaning that the other two could have evolved independently of case building per se.

Although rare, using excretions to thermoregulate is not unknown in insects and these examples may hint at possible thermoregulatory steps through which fecal cases could have evolved. In general, excreting feces that contains some of the body's heat could lower internal T_b, since defecating warm excrement directly removes heat otherwise left in the body. Several insects are capable of evaporative cooling (Kammer 1981, Prange 1996), but evaporative cooling in insects is stifled in part by the nearly impermeable cuticle. Therefore, heat is most often lost with water vapor during respiration (Prange 1996). To aid the process, some insects cover parts of their bodies with liquid excretions to cool down, as do some bees (Heinrich 1993). Applying wet feces to the outside of the body can serve a similar purpose. The sawfly larvae mentioned above apply wet feces to their abdomens to effectively lower T_b (Seymour 1974). Another limit of evaporative cooling is that it requires high volumes of water to be consumed and then lost to be effective (Kammer 1981, Prange 1996). Again, feces-covered sawflies actually exhibit higher levels of evaporation than other species studied (Prange 1996). Numerous species of leaf beetles retain feces in a similar manner; as wet coverings for eggs and larvae (Müller and Hilker 2004). These coverings are often assumed to be antipredatory, but might have initially served to adjust temperature. Perhaps the elaborate fecal cases of the Camptosomata began as a simple way of lowering internal T_b, evolving (possibly to meet other needs) into more behaviorally complicated structures.

Potential costs and trade-offs of casebearing in relation to temperature

The casebearing habit itself potentially poses trade-offs with regard to thermoregulation. Elongate insects usually maintain internal temperature closer to ambient than spherically-shaped ones due to their relatively larger surface area-per-volume, and it can be harder for spherically-shaped insects to thermoregulate by posture orientating, as do dragonflies, grasshoppers, and caterpillars (Casey 1981). The bent over shape of the larval abdomen in Camptosomata and the case itself increase the volume of larvae compared to their surface area, giving casebearers increased heat retention compared to insects of different shapes but comparable mass. Covering their bodies with a dark object might also absorb solar radiation more quickly, potentially raising T_b above optimal and even to lethal temperature, although we found no evidence for this here.

There may also be trade-offs between two, independently beneficial functions of fecal cases, i.e., preventing overheating and preventing predation. If fecal cases are derived from wet feces used as an evaporative coolant, the current hard dry cases would have lost most of this function. Instead, dry, hard cases are better at preventing attack from arthropod predators (Chapter VI). Holding the case down tight to the substrate aids this defensive barrier (Wallace 1970, Root and Messina 1983, Chapter IV), but as we found here, larvae held the case above the substrate when temperatures were high. Larvae facing immediate danger from predators in hot weather may therefore be limited in their ability to effectively cool down while staying defended.

If temperatures on the tops of leaves are such that overheating is likely, then *N. platani* may be restricted to the lower sides of leaves, and thus would be excluded from part of their habitat where they might otherwise find additional sources of nutrients and faster growth rate or areas to escape predators (e.g., Niesenbaum and Kluger 2006). Interestingly, populations of *N. bebbianae,* which feed on Bebb's willow (*Salix bebbiana*), a host plant that ranges across Canada, are more often found on the upper side of leaves than are southern species (Brown and Funk 2005). These populations may be released from the temperature constraint felt by more southerly species and thus simply move about the plant freely. Alternatively, they may be thermoregulating in the opposite direction: moving to the top of leaves to increase their temperature and maintain growth rate and performance (Heinrich 1993, Kingsolver and Huey 2008). Of course, this trade-off is not necessarily confined to casebearers.

Casebearers could actually be free of one constraint often faced by builders. It is difficult for cryptic animals, which rely on limited movement to reduce conspicuousness, to thermoregulate by microhabitat selection. However, organisms that are protected in other ways, such as with aposematic coloration, are free to seek out optimal microclimates (Heinrich 1993). Much architecture allows thermoregulation, such as the tents of caterpillars, moth cocoons, and tiger beetle turrets (Heinrich 1993, Hansell 2005), but these are also immobile and therefore suffer the same problems as crypsis. Insects that build portable architecture, such as fecal cases, may experience the best of both worlds.

Conclusion

It is difficult to determine whether or not a given trait is adaptive. It is also difficult to elucidate which selective pressures are most important in a given situation. Organisms do not live with one selective force only, but must balance abiotic and biotic factors to optimize fitness. Consequently, behavioral and physiological traits can evolve to serve multiple purposes, sometimes one more than others. Here we show that fecal cases might lower the chances that larvae and/or pupae suffer from high temperatures by slowing acclimation time and reducing temperature over ambient when trichomes are incorporated. Fecal cases are also well-suited to reduce successful attack from predators (Wallace 1970, Root and Messina 1983, Chapter IV) and they interact with humidity conditions in ways that affect fitness (Chapter VI). Traits that enhance one function might reduce the effectiveness of another. Here, we show that a thermoregulatory strategy (holding the case above the substrate when temperatures are high) may negate a previously demonstrated antipredatory strategy (holding the case down to prevent attack).

Thermoregulation and predation interact in many species, such as those with camouflaging traits that cannot select microhabitats as readily. Similarly, aposematic organisms often have color patterns and gregarious lifestyles that evolve together to prevent predation (Ruxton et al. 2004), but both of these traits can have great impact on thermoregulation (e.g., Bryant et al 2000, Nice and Fordyce 2006). Hanna and Cobb (2006) found that lynx spiders do not place their egg sacs in temperature locations that would minimize growth time (close to the ground in the open), but rather place them on the apical parts of plants, usually partially sheltered by flowers. Since the mothers need to guard their egg sacs in order to insure egg survival, their choice of oviposition sites allow the mother to feed while simultaneously guarding her brood. Therefore, the authors conclude that predation prevention overpowers the pressure to keep eggs in optimal temperatures.

Although we feel that fecal cases and their related behaviors could be important for thermoregulation in this group, it does not seem as pronounced as the antipredatory function found elsewhere. Fortunately, the fecal-retaining chrysomelids provide a wealth of ecological diversity in which relative selective pressures could be teased apart. In fact, the Camptosomata alone possesses species that live across ecoregions, in open environments (such as *Neochlamisus*) and in very closed ones (such as myrmecophilous clytrines), and which feed on diverse food (herbivores and detritivores). In this first thermoregulatory study, we found a potentially adaptive response of this unique form of animal architecture with respect to a prominent abiotic environmental factor. We cannot fully understand the evolution and implications of fecal cases of Camptosomata without knowing more about many selective forces and their interactions.

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CHAPTER VIII

DEATH BY MOTH: INCORPORATION OF *NEOCHLAMISUS* LEAF BEETLE FECAL CASES INTO SILK BAGS OF THE BAGWORM *THYRIDOPTERYX EPHEMERAEFORMIS.* A BUILDING BUILT WITH BUILDINGS

Abstract

Camptosomate leaf beetles build a unique animal architecture out of their own fecal material to protect their eggs, larvae, and pupae. Lab studies show that these fecal cases help prevent successful predator attack, but few observations of predation in the wild have been reported. Here we describe an unusual, but possibly common, biotic cause of death for one casebearer, *Neochlamisus platani* (Brown 1952). These builders died, not because of actual predation, but because their cases were incorporated into the structure of another builder, the evergreen bagworm *Thyridopteryx ephemeraeformis* (Haworth 1803). Both egg and larval fecal cases of *N. platani* were found sown into bags of seven caterpillars from sycamores (*Platanus occidentalis*) at three locations in middle Tennessee. Fecal cases were intricately woven into multiple regions of the bag material and to different objects in the bag. Of caterpillars that had cases in their bags, an average of two cases were found. One larval beetle was found attached to a bag alive and unable to free itself. We discuss the consequences of this incorporation and other patterns of *T. ephemeraeformis* construction.

Introduction

The Camptosomata are a group of leaf beetles (Coleoptera: Chrysomelidae) that build a case using their own fecal material (Erber 1988). Each case begins as an egg case that ovipositing mothers build out of many small fecal plates molded around each individual egg (Erber 1988, Brown and Funk 2005). Once a larva hatches, it breaks open one end of the egg case, but rather than discarding it, it adds to its mother's construction, increasing the case’s width and length throughout the juvenile stadia. One end of the larval case remains open, allowing the larva's head and legs to be extended for feeding and walking. Eventually this opening is sealed, forming a pupal case. The beetle stays inside the case until the adult cuticle hardens. It then cuts its way out of the fecal material using its mandibles. Usually camptosomates overwinter as adults, which, in the tribe Chlamisini, mimic caterpillar frass (Jenks 1940, Karen 1964, 1972). The current study involves the eggs and larvae of one chlamisine, *Neochlamisus platani* (Brown 1952). *N. platani* is a North American species that mates, oviposits, and feeds exclusively on American sycamore *Platanus occidentalis*. They are univoltine and the adults reach about 3-4 mm in length. Eggs are attached to the host plant by a long flexible stalk. Eggs are typically laid on leaves singly and only occasionally are two eggs found on the same leaf (Hyche 1996, Brown and Funk 2005).

Very few authors have observed predation events of camptosomates in the wild (Donisthorpe 1902, Karren 1964, Neal 1989, Cox 1996, Brown and Funk 2005), however it is not uncommon to find sealed, but empty pupal cases with holes as if attacked by a biting/chewing predator (Flinte and Macédo 2004). Despite these losses, fecal cases protect larvae and pupae against attack compared to when the case is removed (Wallace 1970, Root and Messina 1983, Chapter IV). A more prevalent source of mortality in the camptosomates is parasitoid wasps that somehow bypass the case material to infect their hosts (Brown 1943, Neal 1989, Erber 1988, Brown and Funk 2005). Here, we describe a previously unknown and improbable source of organism-induced mortality that *N. platani* may suffer in the presence of caterpillars of the evergreen bagworm (Lepidoptera: Psychidae) *Thyridopteryx ephemeraeformis* (Haworth 1803).

Psychids such as *T. ephemeraeformis* are also are animal architects. This small brown, somewhat non-descript moth with a very interesting life cycle (Jones and Parks 1928, Kaufmann 1968). As caterpillars, they build a bag by sowing leaf clippings and other gathered materials from their host plant with silk. Pupation takes place inside the bag, which is attached by a thick loop of silk around a branch. Males emerge from the bag at the end of the summer and immediately search for females, which never leave their bags. Females are vermiform, with no (or vestigial) eyes, antennae, wings, or legs, but release pheromones to attract males. Once a male finds a female's bag, he inserts his abdomen, which extends telescopically, to mate with the female, who is sometimes still inside her pupal skin. The female later oviposits several hundred eggs in the bag. Her shriveled body eventually falls out of the case. The eggs overwinter in their mother's bag where they are protected from humidity loss and cold weather (Rivers et al. 2002). Once the caterpillars hatch, they crawl out of the bag and either feed near their mother's bag or drop from it on silken strands. Sometimes they use the strands to catch the wind and the caterpillars "balloon" great distances away from their birth site (Moore and Hanks 2004). Probably due to this form of dispersal and despite the common name, they are capable of feeding on a variety of host plants and are often considered pests (Jones and Parks 1928, Santamour 1980). For instance, the bag's attachment can constrict the branch, forming a gall-like swelling that kills the limb.

Since the two focal species in this work are remarkably similar in many aspects, we try to be very specific in our terminology. When referring to *N. platani*, we use "larva" and "case", whereas "caterpillar" and "bag" will be used for the corresponding components of *T. ephemeraeformis*.

Materials and Methods

T. ephemeraeformis caterpillars and bags were collected from sycamores at four sites in southern Davidson and Williamson Counties, TN on May 27, 2007. They were then brought back to the laboratory at Vanderbilt University in clear plastic bags in a cooler with ice packs to prevent overheating. All bagworms were kept in plastic containers with foliage in a refrigerator until observations were made, nine days after collection. Bags were examined under a dissecting microscope and photographed using a digital USB microscope (Bodelin Technologies, Lake Oswego, OR). Measurements were taken with a digital caliper with an accuracy of 0.03 mm (VWR, West Chester, PA). Length of the bag was measured as the distance from the opening, where the larva emerges, to the bag's narrowed, posterior end. Width was measured at the base (open end) of the bag, which is typically the widest part.

Table 8-1. Details of bagworm bags from sites that contained *Neochlamisus* cases. Bagworm status refers to whether or not the bag was sealed or the caterpillar was active during observations. Size is in milimeters.

Thyridopteryx ephemeraeformis				# *Neochlamisus* cases		
Figure:	Bagworm status	Length	Width	Egg cases	Larval cases	Total
Not pictured	sealed	6.46	2.47	0	0	0
Not pictured	sealed	6.95	3.33	0	0	0
Not pictured	sealed	9.7	4.66	0	0	0
Not pictured	sealed	10.66	4.66	0	0	0
1A	sealed	10.78	3.61	1	0	1
1B	active	12.51	4.12	5	0	5
1C	sealed	10.12	4.4	2	0	2
1D	sealed	6.1	1.61	1	0	1
1E	sealed	8.29	3.5	2	0	2
1F	sealed	9.78	2.95	2	0	2
2	active	11.4	4.63	0	1	1

Results and Discussion

General Findings

Eighteen bagworm bags were collected, but bags from one site did not contain *N. platani* cases and are thus not considered further (n = 7). Bags from three of the four collection sites contained *N. platani* fecal cases (n = 7/11) (Table 1) (Figs. 1 and 2). Two bags contained active caterpillars upon inspection, the rest were sealed. *T. ephemeraeformis* often seal their bags when disturbed or molting (Rhainds et al. 2009)

The bags were primarily built of silk and plant materials arranged so that the small, posterior end were made of what looked like small pieces of flower and bark rather than leaves and were consequently light brown and white in color and scaly in texture (Figs. 1 and 2). The middle of the bags contained bud sheaths and leaf stipules that had dried to brown. The bases of larger bags contained leaf cuttings that could confidently be ascribed to *P. occidentalis* as they included characteristic stellate trichomes (Fig. 2). Smaller bags contained only the white and light brown flower-like material.

The seven bagworm bags that contained *N. platani* fecal cases were 9.34 ± 0.64 mm in height and 3.63 ± 0.30 mm in width on average and were thus probably rather young (probably in the first three stadia judging by length), since seventh instar (= last) caterpillars can be ~50 mm in length (Kaufmann 1968). Bags that contained *N. platani* fecal cases had 2 ± 0.53 on average, but one bagworm had attached 5 egg cases to its bag. The number of *N. platani* cases found in bags is marginally nonsignificantly correlated with bag height (n = 11, $r^2 = 0.27$, $F = 3.38$, $P = 0.099$), hinting that older, larger

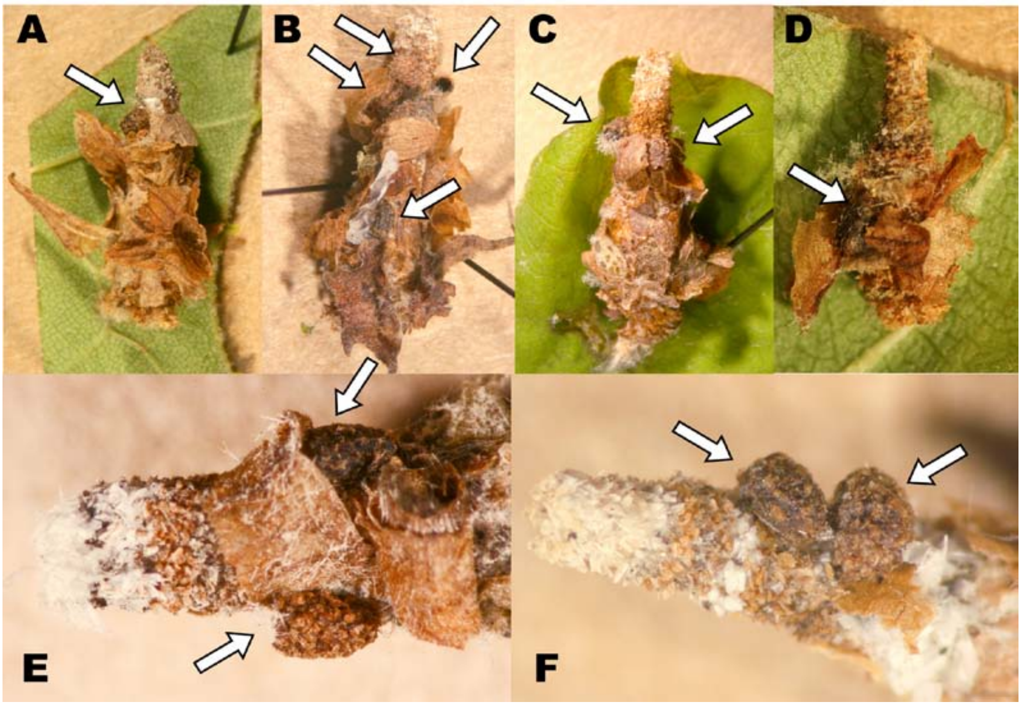

Figure 8-1. *Thyridopteryx ephemeraeformis* bags containing egg cases of *Neochlamisus platani.* Each image depicts a bag from a different individual. An arrow indicates each egg case visible in the picture, but the bag in B contains one egg case on the other side of the bag. Fecal cases are attached tightly along their long axis.

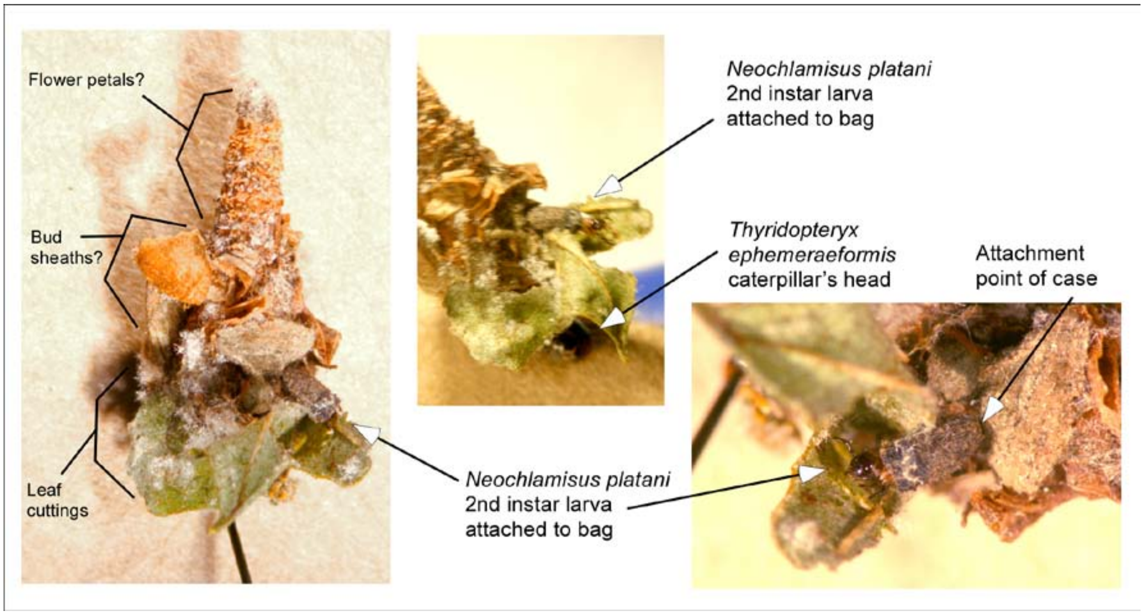

Figure 8-2. A *Thyridopteryx ephemeraeformis* bag containing a larval case and living larva of *Neochlamisus platani*. Only one individual bag is depicted. The *N. platani* larva is attached tightly at the apex of its case. The larva is free to move within its case and is not attached directly to the bag, yet could not dislodge its case and would not leave it. This bag also illustrates the distinctive layers of materials indicating changes in material collected as the bag was built. The apex contains finely shredded particles of bark or flowers, followed by a layer containing mostly bud sheaths or stipules. The base is surrounded by green leaf cuttings.

caterpillars have had more opportunity to incorporate more cases into their bags. However a larger sample size would be more informative.

One bag contained a living (~ 2nd instar) larval *N. platani*, still in its case, that was sown to the bag (Fig. 2). Both caterpillar and larva were still active and were observed for several minutes. The larva was not able to free itself from the bag despite many apparent tries. It would emerge partway out of its case, grab onto a leaf fragment attached to the bag, and pull at its case. Instead of dislodging the case, or leaving the case entirely, it would walk back and forth along the leaf fragment in a failed attempt to move the case.

Implications of case incorporation

Egg cases were tightly sown onto plant material attached to the bag, along one side of the long axis of the case (Fig. 3), rather than simply being attached to it via the egg stalk. So it seems that caterpillars removed each egg from the plant, then attached the egg case directly to the bag, rather than attaching a leaf fragment to their bags that simply happened to contain an *N. platani* egg. This finding removes another possibility, although rather unlikely, i.e. that female *N. platani* laid their eggs on the bags, rather than the egg cases being collected by the caterpillars. However, we did not actually observe caterpillars removing cases and incorporating them into their bags. Egg cases were dispersed throughout the bags, rather than fitting to one region in particular. This indicates that they were added periodically over time or after the majority of the bag was formed, but we are not sure if caterpillars are capable of adding new outer layers to the

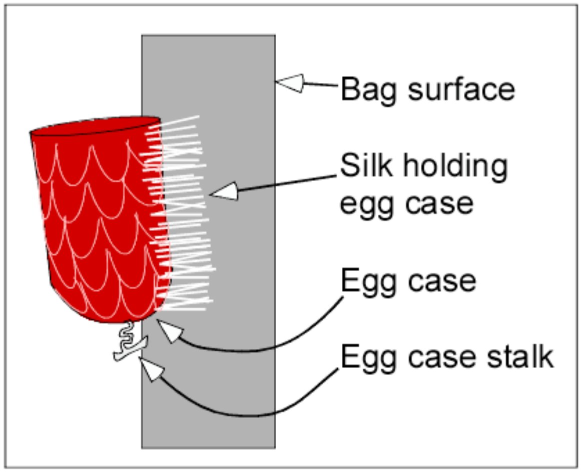

Figure 8-3. Diagrammatic representation of how an *N. platani* egg case is attached by *T. ephemeraeformis* to its bag using silk. Since the egg case stalk, which ordinarily attaches the egg case to the leaf surface following oviposition, is not attached to anything, it seems that *T. ephemeraeformis* intentionally removed the egg case from the leaf and attached it to its bag rather than inadvertently adding a leaf fragment that contained an egg case.

posterior of the bag once built (but see Kaufman 1968 for details on bag construction and repair).

Some eggs still retained the top part of the case (i.e., the egg case roof), which is removed after larvae hatch from the egg and remove the egg case from the plant (Brown and Funk 2005). Presence of egg case roofs indicates that the beetle was inactive when taken. Other egg cases had had their roofs removed and were empty, indicating one of three possibilities: 1) it was taken as an empty egg case from which the larva was already missing, 2) it was taken as a viable egg and the larva hatched and opened the roof after attachment to the bag, or 3) these are actually not egg cases, but 1st instar larval cases, which would have already opened the roof, but not added to the case. If the first, then the larva died of some other cause and the *T. ephemeraeformis* is only a scavenger collecting whatever "junk" it finds and deems suitable. If one of the latter two, *T. ephemeraeformis* caused the death of the larvae, either by feeding on it or by incorporating its case. Some psychid caterpillars are omnivorous and are known to feed on small insects (Rhainds et al. 2009), however most reports state that *T. ephemeraeformis* are herbivorous, although extreme generalists. Some *Neochlamisus* egg cases were taken still closed, and one larva was taken alive, thus these *Neochlamisus* were not eaten by the caterpillars. Therefore, this is not typical "predation" per se, but *Neochlamisus platani* do not survive without their cases (Donisthorpe 1902, Briggs 1905, Erber 1988, Müller and Hilker 2004, Brown and Funk 2005, Chapter III, IV). Since several opened egg cases were found in bags, some combination of these scenarios could have taken place. The bag containing the living larva is evidence for the third option, unless it was incorporated as an egg, hatched on the bag, and then fed and added to its case while attached. Alternatively, the larva might have been undergoing ecdysis in a fixed case and thus was immobile when incorporated into the bag. Otherwise, the caterpillar took it while still active, perhaps after a stuggle.

Examination of a large bag (25 mm) collected previously from an unknown site revealed the incorporation of another, much smaller *T. ephemeraeformis* bag (~6 mm) as well as a *Neochlamisus* egg case. Another bag on a different host contained plant seeds. It seems that *T. ephemeraeformis* has some inclination to incorporate a variety of "found" objects from their habitats, besides basic plant structures like leaves and sticks. And Kaufman (1968) states that bagworms will construct and repair bags out of tissue paper and other given items. It is rather fascinating that the average number of fecal cases found in bags is greater than one since *N. platani* eggs found in this area are typically spaced evenly among leaves (see also Brown and Funk 2005). Do *T. ephemeraeformis* caterpillars deliberately seek out fecal cases in order to attach them to their bags? If so, why? Fecal cases, as well as other solid objects of the leaf surface, may provide some kind of "super cue" to caterpillars that signify much desired building material. Alternatively, the incorporation of fecal cases by *T. ephemeraeformis* might be adaptive for the bagworm. Fecal cases can provide a barrier to arthropod predators (Wallace 1970, Root and Messina 1983, Chapter IV) and other chrysomelid fecal structures have been shown to contain toxins that protect their bearers from attack from predators and parasites (Morton and Vencl 1998, Vencl and Morton 1998, Vencl et al. 1999, Vencl et al. 2005). Therefore, *T. ephemeraeformis* might benefit from the physical and/or chemical barriers provided by fecal cases.

Some thoughts on bag construction

Details of the building behaviors of *T. ephemeraeformis* are few, although the basic design was well described by Jones and Parks (1928) and Kaufmann (1968). It is interesting to see such a distinct pattern in material make-up of bags (Fig. 2). All bags probably begin as a sack of small fragments of bark, since the caterpillars do not move when the case is started (Jones and Parks 1928, Kaufmann 1968). Assuming that the case is built only by adding material along the opening, as do caddisflies (Hansell 2005), then young caterpillars must first incorporate what looks like white petals. As *P. occidentalis* flowers do not have such petals, it seems more likely that they represent the white peeling bark of this tree. Older caterpillars must be responsible for incorporating bud sheaths or stipules into the case, later attaching leaf fragments to the bag. This pattern may reflect plant phenology if bark or flowers, bud sheaths, and leaves are available sequentially in that order. Interestingly, few sticks or twigs, which provide the major building medium of *T. ephemeraeformis* on other hosts (personal observation), were found in the current study individuals, but perhaps these are only added by later instars than evaluated here.

Another explanation for the pattern of building materials in bags would be that particular media are based on changing feeding preferences or locations within or between plants. Jones and Parks (1928) mention that larvae often drop from one part of the plant to another via their silk and they are also known to change individual plants and plant species during development, although rarely. It seems unlikely that the seven individuals inspected for this study would change plants in a sufficiently consistent pattern to account for the highly similar construction of their bags. Therefore the differences in bag make-up within individuals could be due to changes in plant phenology or by changing feeding location within a plant as the bagworms age. Such mobility within a plant makes it easier to understand how the bagworms could incorporate several disparate egg cases. Since *T. ephemeraeformis* regularly feed on 50 or more plant families (Santamour 1980, Rhainds et al. 2009), we wonder if they also incorporate the fecal cases of other species of *Neochlamisus,* which can be found on a variety of coexisting tree and shrub species (Karren 1972), including *Quercus* sp., *Acer rubrum* (Santamour 1980), and *Salix* sp. (Jones and Parks 1928).

Conclusion

Although unusual, it is not unknown for one animal architect to use another architect's buildings for their own constructions. Many birds use spider webs to build their nests (Hansell 1984), barn swallows often attach their nests to the clay nests of wasps (Jackson and Birchfield 1975), and certain caddisfly species that build their cases predominately from the cases of other caddisflies (Boyero and Barnard 2004). However, this study is one of the few documented cases of something, other than parasitoid wasps, "attacking" these beetles in the wild, and the first that we are aware of that mentions fecal cases of the Camptosomata being incorporated into any other form of animal architecture.

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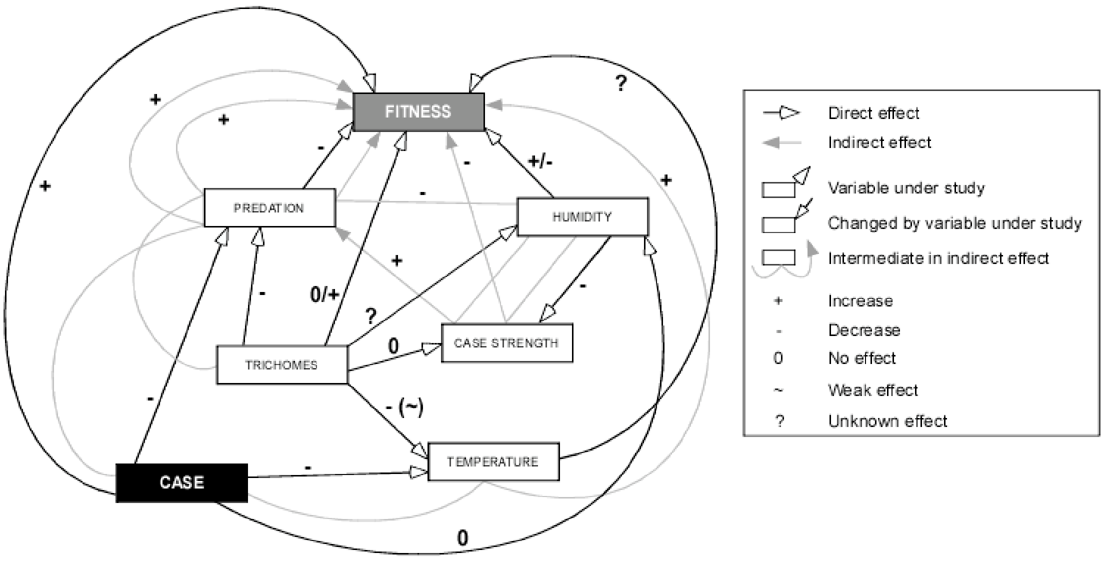
CHAPTER IX

SUMMARY AND RELATED PROJECTS

General Findings

Camptosomate fecal cases have piqued the interests of researchers for well over a century (e.g., Collett 1883,Cockerell 1891, Scudder 1891). These structures are definitely unique among the many diverse building behaviors found in the animal kingdom and even among the fecal-retaining chrysomelids (von Frisch 1974, Olmstead 1994, Müller and Hilker 2004, Hansell 2005). Fecal cases are intricately sculpted both by ovipositing females and later by larvae. They are amazing feats of engineering, given their wet and fragmented material make-up and the malleability needed to expand it, yet cases are durable when dry and their walls are remarkably uniform (Chaboo et al 2008). Almost all authors have assumed that fecal cases protect their bearers from predator attack, among other things, but despite the long-standing interest, these functions have seldom been tested thoroughly (but see Wallace 1970, Root and Messina 1983, Flinte and Macêdo 2004 for smaller-scale investigations). My thesis provides a comprehensive look into the mechanisms by which the case is built and many of the assumed adaptive functions it serves. We can see that many of these features are intricately interconnected (Fig. 1).

I first examined the general natural history and building behaviors of *Neochlamisus* (Chapter II), discovering many temporal and spatial patterns of case construction. During these observations and in a further test (Chapter III), I discovered that fecal cases are required for survival under most conditions, even considerably natural ones. Chapter III shows this importance, but does not point to definite causes. Instead, both abiotic and biotic selection pressures are possible. Later chapters investigate the contributions of various assumed or hypothesized functions in relation to these pressures. In Chapter IV, I tested the effects of larval and pupal cases of two *Neochlamisus* species against three experimental arthropod predators. I found that fecal cases greatly reduce the threat exerted by all of these predators for both larvae and pupae, but not always in the same way. *Podisus maculiventris*, a piercing/sucking predator, were unable to penetrate cases, however larvae are at slightly more risk than pupae, since they maintain an opening in the case through which the bug's proboscis can be inserted. Larvae, however, are better able to escape attacks from the biting/chewing crickets. Pupae are immobile and cannot benefit from the temporary distraction of this predator by walking away. Extra architectural components of the cases of *N. platani* discovered in Chapter II (i.e., external trichomes and trichome attics) were also found to aid survival against crickets, as tested in Chapter IV. The ball of plant hairs embedded in the fecal



Presence of/increase in will increase (+), decrease (-), or have no effect (0) on , either directly (—▷) or indirectly (◄——) through . Some interactions were weak (~) and others were not directly tested (?).

Figure 9-1. Relationships among investigated factors.

cases may help larvae survive attack, while the trichomes interwoven into and onto the case exterior seem to deter attacks in the first place.

These plant hairs, or at least the young leaves where they can be found, are eagerly sought after by *N. platani* larvae (Chapter V), despite a repulsive effect of trichomes on most insects (Levin 1973). Some insects get entangled by trichomes or they cannot feed on pubescent leaves. *N. platani* larvae however can maneuver over them when young and through them when older. They either cut them from the leaf surface or devour them whole. Through either mechanism, trichomes become incorporated into and onto the case, where they then serve their protective function. Thus, *N. platani* presents a unique situation in which a physical plant defense has been co-opted by an insect that feeds on it.

In Chapters VI and VII, I examine the role fecal cases of *Neochlamisus* play in moderating the effects of abiotic conditions, a function less often stated in the casebearer literature, but very common for other animal architectures (Danks 2002, Hansell 2005). Many insect constructions protect their builders from desiccation (Silverman et al. 1981, Hieber 1992, Tagawa 1996, Zamora-Munoz and Svensson 1996, Rivers et al. 2002), a condition most often faced by small organisms (Casey 1981), and I tested this role using pupal cases of *N. platani* in Chapter VI. I found that pupae with their cases removed did not suffer any loss of weight in low humidity conditions or gain of weight in extremely high relative humidity as expected when compared to pupae with their cases. I also found that pupal cases did not increase survival to adulthood in low humidity treatments. Instead, I found that pupae without cases are equally likely to survive in relative humidity of ~30%, despite massive larval death found by myself (personal observations) and other authors (Karren 1964, 1972, Boldt and White 1992, Olmstead 1994, Flinte and Macêdo 2004, Chaboo et al. 2008) in relatively benign laboratory settings and in my own field studies (Chapter III) when the case is removed. Perhaps pupae are better able to withstand these conditions than larvae. Even more shocking, I discovered that fecal cases left intact were actually devastating to beetles in low relative humidity. Decreasing humidity increases case strength, which, although beneficial in the presence of predators because it increases a predator's handling time, strong cases are impossible for adults to cut their way out of (Fig, 1). Instead, when reared under low humidity, the adults develop normally, but die inside their cases.

In Chapter VII, I investigated the role of fecal cases and associated behaviors in relation to temperature regulation. I found that *Neochlamisus* may be most adapted, through fecal case structure and behaviors, to prevent overheating. Fecal cases were found to slow the rate at which internal temperature reaches 40C when compared to not having a case. Adding trichomes to the case also maintained temperature below ambient when ambient reached more than about 30C. The larvae themselves expressed behaviors that may serve the purpose of lowering temperature. They spend the majority of their time on the undersides of leaves, where they experience cooler temperatures than the tops of leaves, and when the temperature is high, larvae often raised their cases above the leaf surface so that their legs were showing. Raising the case may allow better airflow or lift the larvae above the leaf's boundary layer to where temperatures can be cooler. Normally, the case is held tight to the substrate when resting and feeding, which has been shown to limit attack success from numerous predators (Wallace 1970, Chapter III). In hot temperatures however, larvae may need to forgo that measure of safety in order to cool down.

As stated above, all of these aspects of fecal cases are intricately interwoven (Fig. 1). Presence of a case greatly increases survival directly (Chapter III) and indirectly by reducing predation (Chapter IV) and perhaps for larvae by maintaining some abiotic factor (Chapter III), but does not increase survival in low humidity unless predators are present (Chapter VII). Being reared on pubescent leaves might affect fitness directly, by increasing larval weight and reducing development time, which is not in the expected direction given the difficulty plant hairs evoke for other herbivorous insects. Trichomes also affect beetle survival indirectly: Trichomes in the cases of *N. platani* lower the maximum temperature obtained (Chapter VIII) and reduce the threat from biting/chewing predators (Chapter IV). Trichomes do not increase case strength, but case strength also greatly increases predator handling time (Chapter VI), which would increase survival and fitness. If cases are hard at the end of pupation, though, they can be too strong for adults to emerge from, which is very bad for fitness (Chapter VI). Humidity affects beetle survival directly since larvae need some moisture to survive, although too much causes mold, at least in the lab. Humidity also affects fitness indirectly by affecting case strength (Chapter VI). The association between humidity and case strength is positive in some circumstances and negative in others. The affect of external trichomes on internal humidity is an obvious question, but was not investigated here. These interactions provide a complicated framework of pushes, pulls, directional selection, and trade-offs in which the casebearing habit must have evolved and is currently evolving.

Chapter VIII highlights a unique observation I made while collecting *N. platani* for experiments. Few observations of camptosomate predation have been reported in the literature (Cox 1996, Erber 1988), despite common appearances of torn pupal cases, the apparent result of predation (Flinte and Macêdo 2004). On a routine collecting trip, I found not one, but several, bags of the evergreen bagworm moth *Thyridopteryx ephemeraeformis* that contained egg and larval cases of *N. platani* as part of their material make-up. Cases were sown into the bags along the long axis of the case. Despite the relative sparseness of *N. platani* eggs on leaves, several bags had multiple egg cases in them, indicating that *T. ephemeraeformis* sought out these structures for their own bags. Although I could not determine if the eggs were viable when collected, one bag contained a larval case with a living larva still inside. This poor creature was able to move its legs along a leaf fragment also attached to the bag, but was unable to remove its case. This is a rare observation of an animal architect using the buildings of another architect in their own constructions. Even more infrequent, this may be the only example of a beetle dying at the "hands" of a moth in the wild (see Pierce 1995).

Related Projects

Chaboo, C, CG Brown, and DJ Funk. 2008.

Faecal case architecture in the *gibbosus* species group of *Neochlamisus* Karren, 1972 (Coleoptera: Chrysomelidae: Cryptocephalinae: Chlamisini). *Zoological Journal of the Linnean Society*. 152: 315–351.

A related project, not included as a chapter, is a recently published collaboration with my advisor and Caroline Chaboo that details the different structural components of cases across life stages of several *Neochlamisus* taxa with many accompanying pictures and SEM (Chaboo et al., 2008). It is the first paper to specifically describe fecal case structure in such detail and across so many taxa. The discussion of this paper provides a practical guide and glossary to facilitate similar comparisons in other groups. It also begins to make these comparisons by reviewing case descriptions found in the literature. It treats individual components of fecal cases as morphological characters and hopefully will aid understanding of how such a complex behavior evolved and some of the pressures molding it through ecology and over time. We hope to see more phylogenies that help to determine the evolutionary patterns of fecal use within the chlamisines, camptosomates, and chrysomelids (as in Fig 1, Chapter I).

We found that cases across *Neochlamisus* taxa are surprisingly similar and differ mostly in size, color, and trichome incorporation, while also differing modestly in shape. Development of cases across the genus seems to follow the building behaviors described in Brown and Funk (2005, Chapter II). All species begin the case by building an egg case of overlapping plates, although the shape of the plates and the presence of finer details, such as plate ridges and a flange around the roof of the egg case, differ across species. All larvae add new material to their cases in a similar fashion, both by adding horizontal rows to the dorsal rim and by cutting and expanding the ventral suture. This change in pattern, along with the attachment of larval additions to the maternal egg case, is very prominent when viewed in high magnification SEM. The length of the ventral suture, however, differs somewhat and may explain the presence or absence of a "nipple" on the apex of the case, a trait considered by Brown (1943) to be distinctive and phylogenetically relevant between *Neochlamisus* and the closely related *Exema*.

Trichomes were embedded in the fecal matrix of the case walls of all *Neochlamisus* taxa studied, but the role of external trichomes may only be significant in three taxa. The quantity and importance of trichomes in a given beetle species most likely relates to its host plant, but it is unknown if the trichome-related preferences and behaviors of *N. platani* are shared by other *Neochlamisus* species. In this paper, we suggested that *Neochlamisus* might embed trichomes in order to maintain the structural integrity of the case. My test of case strength however, found no difference between cases of larval *N. platani* reared on foliage with and without trichomes (Chapter V), although there are many aspects of case strength and the type of manipulation it is designed to withstand (e.g. stress, strain, compression, and tension) and only one type of test was performed. Trichomes might also negate some of the changes in case strength during extreme changes in humidity, such as case weakening when humidity is high.

Cross sections of case walls showed that the wall structure is impressively uniform in thickness across the circumference of the case, with the exception of a thinner line at the ventral suture (see Fig. 9 in Chaboo et al. 2008). This area may be kept thinner intentionally to enable case cutting and subsequent expansion or it may be a necessary consequence of building the wall from an internal position. The inside wall facing the larval chamber is rather smooth, while the outside is very rough and irregular. The inside wall rarely contains any trichomes projecting into the chamber even when the outside is pubescent and there are trichomes throughout the thickness of the wall itself. How do larvae keep the wall so smooth? There are probably multiple layers of fecal material on the wall, although this is not readily obvious from the cross sections. I would suppose that larvae add coatings to the inside after constructing the wall out of rows in order to smooth the inside or to add strength (as do wasps, Wenzel 1993). Trichomes have been found in the guts of dissected *N. platani* larvae (Chapter V) and thus are likely to become mixed with the fecal material of the case when excreted (Chapter V). Larvae may be able to control when trichomes are added to new material by controlling their feeding or excretion. Perhaps they feed on areas with fewer trichomes or shave the leaf of their trichomes before adding smoothing layers. Obviously, many behaviors used in building fecal cases have yet to be explored.

Building gnomon

Biological structures often need to grow in size without changing shape. Several animal architectures are built in gnomonic fashion as material is added in simple, repeated patterns in one direction. One building pattern that facilitates these constraints is a cone, which becomes a larger cone of the same proportions when material is added to the growing edge at a constant angle (Hansell 2005). Some caddisflies choose sand grains of a certain size to add to their cases and the size grain chosen increases gradually over time, imparting a constant growth curve, despite the fact that caddisfly body size does not change gradually, but only at molts (Hansell 2005). According to Chapter II, *Neochlamisus* add to their cases continuously as larvae, not just prior to molts, as hypothesized by Erber (1988). Are *Neochlamisus* using a template to build the case and determine its size? Case shape, however, is not constant. Fourth instar larvae ‘round out’ their cases in preparation of pupation (Chapter II). Similarly, many caddisfly larvae switch case design and technology at a certain point mid-development, indicating that building behaviors are not simply stereotyped and conserved throughout the building process (Hansell 2005).

One way to add volume while limiting surface area is to build in a spiral, such as in mollusk shells. This type of spiral, the logarithmic spiral, is a gnomon found in very diverse taxa from sunflowers to ram horns (Vogel 1998). Logarithmic spirals are also known in caddisfly cases of the Helicopsychidae, another structure built by an insect larva (Hansell 2005). Anthony Aldridge (1998) showed that brachiopod shells, although not a complete spiral, grow logarithmically as the animal adds material in simple, repeated increments to the outer rim of the shell. *Neochlamisus* cases start off as straight-walled structures (the egg case) but later become more curved toward the substrate as larvae add to the dorsal rim more than the ventral rim (Erber 1988, Brown and Funk 2005). I hypothesized that *Neochlamisus* also add to their cases logarithmically. I contacted Anthony Aldridge and sent him a photograph of a particularly round *N. bebbianae* pupal case. He inserted the outline of the case into software that mapped its coordinates and compared it to a logarithmic spiral (Fig. 2). He said that this growth pattern is a reasonable explanation for case shape. He also said that the case may be formed in multiple spirals over time, possibly as the larva grows or as a function of the

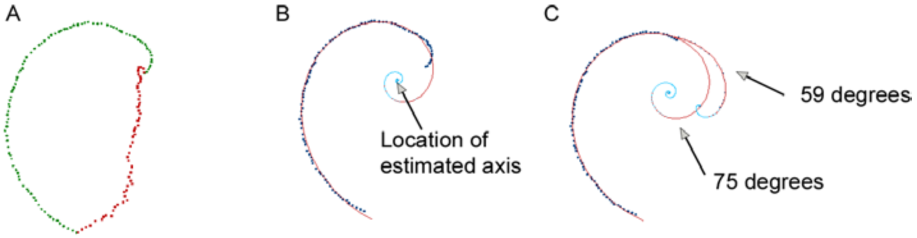


Figure 9-2. Proposed logarithmic spiral pattern of fecal cases. A. Outline of *Neochlamisus bebbianae* pupal case digitized as points (= coordinates on a grid). B. Dorsal edge fitted with a single spiral continued to axis to show overall shape. Spiral is 72 degrees where 90 degrees is a circle. The overall fit is poor, especially at the location of the maternally -built egg case section near the apex of the case. C. Outline fitted with two separate spirals, showing a much better fit.

maternally-built section (Fig. 2), as in the brachiopods he studied previously (Aldridge 1999).

Case strength is probably enhanced by its shape. Round shapes and domes are stronger than their two-dimensional counterparts. For example, a flat sheet of paper cannot stand up when placed on its end, but if curved into a tube, it can support quite a bit of weight before being compressed. If the case is built logarithmically, this pattern not only increases interior room while limiting surface area, it may also increase the strength of the case or offer better weight displacement. This discovery provides more insight into the building patterns used by larvae to build their fecal cases and shows that their template may change over time. Case shape may also enable building methods to be stereotyped and simple, i.e. by requiring additions only to one side and keeping the same rules, despite growth. It could explain some of the cues and constraints used to build the case, and further exemplifies the evolutionary differences between fecal cases and the other forms of fecal retention found in Chrysomelidae, however it speaks to convergences in building strategy between this structure and more disparate animal architectures such as caddisfly cases and snail shells.

Unanswered Questions

As mentioned throughout this dissertation, many questions remain to be investigated. The cues and constraints impressing on the development of fecal cases within the Camptosomata are largely unknown. Most casebearing species are poorly described or studied, the taxonomy has changed many times, and the terminology used to describe fecal cases in the literature is not consistent. There are few descriptions of case form and fewer on larval behavior. Of course, the myrmecophilous lifestyles of some species in Clytrini and Cryptocephalini make this even more challenging. There are currently no molecularly-based phylogenies comparing species within the camptosomates and their relationship to the rest of the leaf beetles is vaguely understood (see Fig. 1, Chapter I).

Many aspects of the predatory pressures faced by Camptosomata are unclear. Some authors assume bird and ant predation, but no observances of this in the field have been indicated. A visual predator is possible, especially given the open-feeding habits of the phytophagous casebearers and the apparent mimicry of adult chlamisines, although vespid wasps may be more likely (Schenk and Bacher 2002). See Chapter III for more potential predators. If a visual predator, such as birds, is discovered to play a major role, we might hypothesize that fecal cases and trichome additions help camouflage immature beetles, which should be tested with appropriate predators and with larvae and pupae against a natural background. Thus far, no experimental tests have been performed in a natural setting, expect for Chapter III in this document and Flinte and Macêdo's (2004) moderate examination of *Fulcidax monstrosa* larval survival. The importance of *T. ephemeraeformis* incorporation on survival of camptosomates is also intriguing (Chapter VIII). Are they really seeking out fecal cases for their bags? How widespread are these behaviors?

Almost all camptosomate species brought back to the lab are known to suffer from parasitoid wasps (Brown 1943, Erber 1988, Neal 1989, Olmstead 1994, Schöller 1999). Several of these wasps have been keyed to species, but not much else is known of them (Erber 1988). Are they specialists on beetle larvae? On casebearers? It would seem that some specialization is required to bypass the case, which can block much larger predators, but many wasps parasitize gall-formers and other hard-shelled insects. Only Schöller's (1999) study on one cryptocephaline describes how the wasps attack and emerge from cases. There are two ways in which they can escape the case, either by walking out of the larval opening or by cutting a small circle out of the case wall. Are parasitoids attracted or deterred by the fecal barrier? Are the wasps also host plant specific, as are most *Neochlamisus* species, following their host? Many parasitized larval *Neochlamisus* stop development at the 2nd-3rd instar stage, but only after the case is attached to the leaf surface. Is this timing induced or only exploited by the wasps?

Many studies have investigated the chemical components of tortoise beetle shields and criocerine fecal coverings (e.g. Vencl and Morton 1998, Vencl et al. 1999, Müller and Hilker 2003), but nothing is known of the chemical make-up of fecal cases, except the confirmation that they are made of feces and plant matter over a century ago (Donisthorpe 1902, Briggs 1905). Many leaf beetle species use plant-derived toxins to deter predators. Do the Camptosomata also sequester chemicals from their host plants for their own protection? Are these chemicals more likely to be found in the fecal cases than the larval body? Are there any chemicals, plant-derived or not, that specifically prevent microbial infection? What is the glue that holds cases together? What is the white stringy substance that holds the case to plants during ecdysis?

The importance that relative humidity plays in beetle survival also raises many more related questions. For example, how quickly does fecal cases strength respond to humidity conditions? Is one afternoon of rain or one morning of dew sufficient to enable the adult to escape? What about other species? Many *Neochlamisus* species live in the desert of the southwestern United States. Have they timed their emergence to the period of heavier rains at the end of the summer? What about the species that pupate inside ant nests? Maybe the conditions there are sufficient or perhaps they have softer pupal cases. This tight relationship between beetle survival and environmental conditions may become especially relevant as human-induced changes in weather patterns become more prevelant.

Conclusion

The fecal case of *Neochlamisus* is a remarkable feat of animal architecture. It is apparently required for survival. Without it larvae become weakened in the lab and cannot survive in the field. It may help them keep from drying out, it blocks attack from predators, and prevents overheating. Females require a delicate touch to construct a safe haven for their most precious constructions. Larvae are able to add plant materials to further its protective abilities and perhaps to enhance its stability. Pupae are completely sealed inside their own personal suite for the rough transition between juvenile and adult. With the outside stern, the inside can be smoothened for a comfortable fit. After all of this, poop may not seem so bad.

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