Chapter 15
Symbioses with Microorganisms

Microorganisms are an inescapable presence in most biotic interactions, and they influence the nutritional ecology of natural enemies in at least two major ways. First, their interactions with the food items themselves often change the quality and attractiveness of these substances for natural enemies. Presented in this chapter are three such interactions: when microorganisms (especially fungi) affect seeds, nectar, and honeydew for natural enemies. The microbial community of insect guts plays an important and often underestimated role in the nutritional ecology of entomophagous species, and internal nutritional symbionts are the focus of the second half of this chapter. Clearly, as a discipline we are only just beginning to understand how microbes render the nutritional ecology of entomophagous species more complex, and it is hoped that this short review will stimulate more research in this expanding area of biology.

15.1 Contaminated Non-Prey Foods

15.1.1 Endophytes and Seeds

> *Want ye corn for bread?*
> *I think the Duke of Burgundy will fast*
> *Before he'll buy again at such a rate.*
> *'Twas full of darnel: Do you like the taste?*
>
> Henry VI: Act III, Sc. 2

This passage by Shakespeare describes darnel (*Lolium temulentum*), a plant often found within cereal fields that is best appreciated for its mammalian toxicity and bitter taste. Leemann (1933) presents a compelling case that the toxicity of this plant stems not from the seed itself, but rather from endophytic microorganisms that produce defensive chemicals. Although Leemann believes the endophyte to be

J.G. Lundgren, *Relationships of Natural Enemies and Non-Prey Foods*,
Progress in Biological Control 7,

Published by Springer Science+Business Media B.V.

a fungus, more recent research suggests that a nonfungal endophyte may be responsible for the observed toxicity (Faeth, 2002). Indeed, endophytic microorganisms, particularly fungi, are quite widespread in plants, and the functions of these often mutualistic (from the plant's perspective!) symbionts are being revealed.

15.1.1.1 Patterns in Endophytic Infections

Endophytes by definition live internally within the plant, but are not pathogenic to the host in a traditional sense. Evolutionarily, these endophytic mutualists are likely derived from plant pathogens (Clay and Schardl, 2002). Both bacterial and fungal endophytes are known, although the insect-resistant properties are better appreciated for the fungi. Nevertheless, endophytic bacteria can be quite abundant within plants (Vega et al., 2005), and the plant-insect relationships of bacterial endophytes merit further attention. Endophytic fungi generally stem from the Ascomycota, but are widespread throughout this order (Carroll, 1988). Most endophytes are transmitted horizontally, and are not contained in the embryo of the seed (Faeth, 2002). Exceptions to this are the fungal endophytes of grasses, of which some are passed vertically to the seeds (Faeth, 2002).

The fungal endophytes associated with grasses have been the target of substantial research efforts, in part due to the importance of cereal crops and rangeland forage, and the utility of these plants as turfgrasses (Brem and Leuchtmann, 2001; Clay and Schardl, 2002). The best understood endophytic fungi reside within the Clavicipitaceae (Clay, 1988), which form very close relationships with their grass hosts, nearly attaining organelle status in some cases (Clay, 1992; Clay and Schardl, 2002). One estimate is that 30% of pooid grasses harbor fungal endophytes (Faeth, 2002). In grasses, endophytes display one of three classes of reproductive strategies, (1) exclusive sexual reproduction, (2) exclusive vegetative reproduction, or (3) a little of both (Clay and Schardl, 2002). The endophytes that reproduce sexually are infrequently found in the seeds, and produce fruiting bodies. These species either prevent or reduce seed production in the plant, and so are better described as pathogens. The species that reproduce asexually are dependent on the host plant for their persistence, and are transmitted vertically to the plant's progeny via the seed. Although genetic recombination does not occur in these asexual species, they can hybridize with other strains. The beneficial qualities of endophytes are best appreciated for these asexual forms of endophytes of grasses (Clay and Schardl, 2002; Faeth, 2002; Hill et al., 2005), particularly for those in the genus *Neotyphodium*.

Although endophytes are widespread within the plant kingdom, there is considerable variability in the infection rates and within-plant distribution of endophytes. It is frequently the case that different populations of the same plant species have very different endophytic infection rates (Jensen, 2005). Within plants, many endophytic fungi live intercellularly and are present at different levels in the various tissues. In one case, the endophytic fungus, *Phomopsis casuarinae*, of *Casuarina equisetifolia* is present throughout the entire plant, except for the cotyledon and embryo (Bose, 1947). But the hyphae of the fungus are in the testa of the seed (Bose, 1947). In addition to variability in the infection rates within and among plants, the level of defensive chemicals produced by these endophytes is extremely plastic (Clay and Schardl, 2002; Leuchtmann et al., 2000).

15.1.1.2 Endophyte Function

One way that endophytes benefit their host is through the production of mycotoxins that are allelopathic to other plants, phytopathogens, and herbivores. In particular, many endophytes produce alkaloids (loline, lolitrem, and ergot peramine) (Clay, 1988; Clay and Schardl, 2002), normally not present in aposymbiotic grasses. Mycotoxins produced may also reduce plant competitors and microbiological pathogens. One test shows that 10 of 18 tested grass species produce alkaloids, presumably because of their endophyte mutualists (Leuchtmann et al., 2000).

Although not all grass endophytes alter feeding by herbivores (Saikkonen et al., 1999), several instances where endophyte infections have a strong influence on herbivore–plant interactions are in the literature. In one study, 9 of 11 endophyte-infected grasses have some inhibitory effects on insect feeding (Clay and Schardl, 2002). In one case, herbivores (mammalian and insects) affect the level of infection in tall fescue grasses under field conditions (Clay et al., 2005). There are often reproductive costs and reductions in host competitive ability inherent in endophyte infections (even in asexual grass endophytes) (Clay and Schardl, 2002; Richmond et al., 2003), but these costs are overcome by the contributions to plant fitness made by the endophytes. Thus, the endophyte-plant relationships persist (Clay, 1988; Clay and Schardl, 2002). Still, the interactions between endophytes and plants are complex and the ability of endophytes to reduce herbivory is by no means universal within plants (Faeth, 2002).

Endophytic microorganisms influence granivory of grasses by entomophages, which is understandable given that grasses are the only plants known to transmit their endophytes vertically to their offspring. The alkaloids produced by endophytes are present at their highest concentration in the seeds of ryegrass (*Lolium perenne*) (Ball et al., 1997), and in tall fescue, *Festuca pratensis* (Justus et al., 1997) (Fig. 15.1). Within the seed, the embryo of *F. pratensis* has two fold higher alkaloid contents compared with the rest of the seed (Justus et al., 1997). Endophytes of grass seeds reduce seed consumption by *Pogonomyrmex rugosus* and *Acheta domestica*. In *P. rugosus*, the ants collect infected and uninfected seeds equally, but discard the infected seeds to their middens, which incidentally are an excellent site for germination (Knoch et al., 1993). The endophyte *Acremonium loliae*, in association with ryegrass, is toxic to *Acheta domestica* (Ahmad et al., 1985). Essentially, the mycotoxins produced in the seeds interfere with the membrane permeability of the midgut epithelium, resulting in the dissociation of the gut lining from connective tissues. Ultimately, complete digestive failure occurs in the crickets, which soon die. Given that the seed is such a critical point in the life stage of the plant and that endophytic toxins are expressed at their highest levels in the seeds of grass species (Knoch et al., 1993), it is surprising that more interest isn't given to the importance of endophyte defenses against post-dispersal granivory.

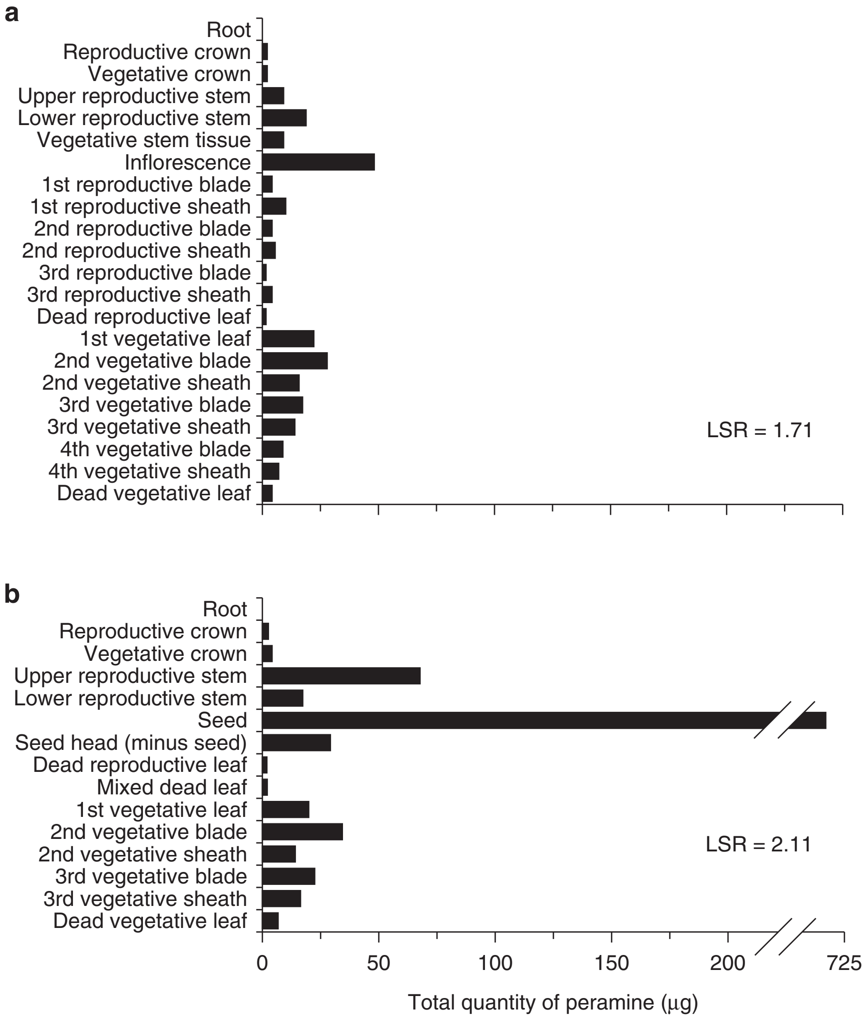

Fig. 15.1 Total quantities of peramine in different components of vegetative and reproductive tissues from endophyte-infected (*Neotyphodium lolii*) *Lolium perenne* plants (Reproduced from Ball et al., 1997. With permission from Springer)

15.1.1.3 Other Seed–Microbe Interactions

Finally, many non-endophytic fungi influence granivory rates by entomophagous insects. For example, seeds uninfected with fungus are removed at twice the rate of moldy seeds by the harvester ant *Pogonomyrmex occidentalis* (Crist and Friese, 1993). Also, some fungal associates may actually make seeds more attractive to granivorous entomophages. Grain infested by fungi may be more easily digested by natural enemies (Dicke, 1988a). Finally, ergot infections of *Paspalum dilatatum* render the seeds more attractive to the fire ant, *Solenopsis invicta* (Vinson, 1972). This is likely because the fungus produces a sugary secretion to which the ants are attracted (Vinson, 1972). The magnitude of interactions of microbial seed associates and granivory rates are still poorly understood, but could be very important to the ecology of both parties.

15.1.2 Nectar and Yeasts

> *It is a fact, which does not appear to be widely known, that the nectar of some flowers is frequently infected with one or more species of yeast, sometimes to such an extent that it is visibly fermented.* (Betts, 1920)

To think of nectar nutrition in the absence of microbes provides an incomplete view of things. Most floral nectars have a diverse microbial community, especially containing yeasts in the genera *Candida*, *Torulopsis*, and *Metschnikowia* (Ehlers and Olesen, 1997; Eisikowitch et al., 1990b; Lachance et al., 2001; Last and Price, 1969; Phaff et al., 1966; Sandhu and Waraich, 1985). In all, dozens of yeast species are isolated from nectar (Last and Price, 1969; Phaff et al., 1966); the nectars in one survey contain 36 yeast species from 12 genera (Sandhu and Waraich, 1985). It might be expected that yeasts should be quite pervasive in floral nectar (Phaff et al., 1966); after all, nectar presents a rich nutritional source for yeasts, and flower-visiting insects provide an excellent mechanism for transferring yeasts from flower to flower (Betts, 1920; Corbet et al., 1979; Eisikowitch et al., 1990b; Phaff et al., 1966). For example, in their examination of the yeasts associated with cactus flowers, Lachance et al. (2001) describes the strong associations between beetle pollinators and *Candida cleridarum*; 19 of 22 collected beetles carried the yeast.

The reality of the situation is that yeasts in nectar are not as ubiquitously present in flowers as one might expect. Although up to 90% of flowers in some habitats have yeasts (Eisikowitch et al., 1990a), and single flowers can support millions of yeasts (Phaff et al., 1966), typical yeast-nectar associations are somewhat less impressive. In most situations, far fewer (usually less than 50%) flowers are inhabited by yeasts (Ehlers and Olesen, 1997; Gilliam et al., 1983; Phaff et al., 1966; Sandhu and Waraich, 1985), and some flower species are entirely devoid of microbial inhabitants (Gilliam et al., 1983). The microclimate found in the flower, presence of antimicrobial secondary chemicals present in the nectar, and the efficiency of transmittal may all contribute to why some flowers have a rich microbial community, while others are practically pristine.

Microbial residents of nectar play an important role in the dynamic exchanges between flowers and insects. From a flower's perspective, troublesome nectar-inhabiting yeasts may reduce self pollination by inhibiting pollen germination, as seen in *Asclepias syriaca* and *Metschnikowia reukaufi* (Eisikowitch et al., 1990b). But, insects with greater residence times on a flower and that fly shorter distances once they have picked up a flower's pollen make the best pollinators from the plant's perspective.

Thus, when some nectar-microbes ferment nectar sugars into alcohols, they cause a ‘drunken pollinator’ syndrome that may promote more efficient pollination (Betts, 1920; Corbet et al., 1979; Ehlers and Olesen, 1997). Ehlers and Oleson (1997) implicates a *Cladosporium* species as the ethanol-producing yeast found in nectar of *Epipactis* flowers that both intoxicates and is transferred from flower to flower by fruit wasps. From the insect’s perspective, yeasts can alter the odor and concentrations of nectar as well as its nutritional composition, possibly affecting its attractiveness and suitability for glucophagous species (Corbet et al., 1979; Kevan et al., 1988). However, one of the only studies to examine this theory found that foraging bees are unaffected by the presence of microorganisms found in *Asclepias syriaca* nectar (Kevan et al., 1988). Regardless, it appears that glucophagous entomophages regularly consume yeasts along with sugar-meals, and the implications of this inadvertent mycophagy for the physiology of glucophagous entomophages remains to be examined.

15.1.3 Sooty Molds and Honeydew

Microorganisms affect the nutrition and attractiveness of honeydews in several ways. First, endosymbionts of sternorrhynchans alter the sugar content of honeydew before it is secreted. More specifically, bacterial symbionts synthesize some of the trisaccharides frequently encountered in honeydews (Bates et al., 1990; Davidson et al., 1994; Tarczynski et al., 1992). As discussed in the glucophagy section, these trisaccharides have important implications for the interactions with natural enemies. Once the honeydew enters the environment, it soon is colonized by a broad microbiological community, the most conspicuous of which are termed sooty molds (Hughes, 1976).

> *The term sooty mould has been and continues to be variously applied. In its broadest sense it has included not only superficial saprophytes but also certain parasitic fungi whose dark, conspicuous, superficial hyphae insert a variety of absorption mechanisms into the plant tissues.* (Hughes, 1976)

All sooty molds are members of the Ascomycetes; other fungal groups do not possess the same pigmentation characteristics present in the sooty molds. Although best appreciated for their symbiosis with honeydew, these fungi also occur in association with other sugar sources like those from glandular trichomes and EFNs (Hughes, 1976). Sooty molds affect the nutrition of honeydew-feeding natural enemies in at least two ways. First, once established, the sooty moulds alter the nutritional landscape of the phylloplane by exuding sugars, pectic acids, and sugar alcohols (Hughes, 1976). Also, the entomophagous species that consume honeydew also eat the sooty molds alongside the sugars. Sheldon and MacLeod (1971) describe the fruiting bodies of the sooty molds, *Helminthosporium*, *Alternaria* (mostly), *Piricauda*, and *Fumago* in the guts of *Chrysoperla carnea* and *Chrysopa nigricornis* collected in the field. Coccinellids are another natural enemy that frequently has sooty mold in its gut. At least 26 coccinellid individuals (many of which were *Coleomegilla maculata*) from one survey ingest spores of

Alternaria, a sooty mold found in peach orchards (Putman, 1964). Although this may be incidental consumption along with the desired honeydew, data from Triltsch (1997, 1999) indicate that coccinellids are more mycophagous than many of us give them credit for.

In fact, feeding on fungi associated with honeydews may have pre-dated actual entomophagy of sternorrhynchan prey that produce the honeydew, and may have led to the evolution of dietary specialization in predaceous beetles, especially within the Coccinellidae (Leschen, 2000). Briefly, those clades that were ancestrally predaceous have not specialized on aphids. But many predaceous beetles with mycophagous ancestors have tended to evolve into aphid specialists. In examining feeding records and phylogenies, Leschen concluded that specialization on sternorrhynchan prey from mycophagous ancestors has likely occurred in 10 out of 11 specialized predatory beetle groups. Thus, sooty molds may have helped to form the current suite of aphidophagous predators.

15.2 Nutritional Symbionts of Entomophagous Species

> *For the most part it was some insufficiency in food sources which led to the establishment of symbiosis, or better stated, certain food sources became available to the animals only after they had symbionts at their disposal to compensate for the deficiencies.* (Buchner, 1965)

Microbial symbioses contribute to the nutrition of an arthropod when (1) the diet of the arthropod lacks specific nutrients entirely, and (2) when required nutrients are present in the diet but are unavailable because of a lack of metabolic tools in the insect or when the nutrient is bound to indigestible compounds. Given that at first glance natural enemies consume nutritionally robust foods for at least part of their lives, it is easy to understand Buchner's early proclamation that predatory species are disinclined toward nutritional symbioses

> *…types of symbiosis which play a role in the metabolism of animals are lacking, above all, in predators…*

But as is repeated in the book you are reading, the diets of arthropods are much less defined than many would prefer. Non-prey foods are extremely heterogenous in their nutrition and defense, and these traits restrict which organisms can consume them. Understanding how nutritional symbioses contribute to the acceptance of non-prey foods by entomophagous species will undoubtedly advance our understanding of how ordinarily carnivorous organisms can make a living as vegetarians, and ultimately how these mutualisms have driven, or at least facilitated, the evolution of herbivory within arthropods. Still, most research only scratches the surface as to how nutritional symbionts contribute to facultative phytophagy in entomophagous species.

A tremendous diversity of microorganisms have formed tight relations with arthropods, including plasmids, protozoa, bacteria, yeasts, and higher fungi (Campbell, 1989, and numerous references therein). Insects are often born with sterile guts, and consume a range of microbes during their lifetimes (Chapman, 1998). Koch (1960) states that

> *Without doubt, the majority of symbionts were first taken up with nourishment by way of the mouth.*

Thus, when the microbial community of an insect's gut differs from that of the surrounding habitat, it may be worth exploring for mutualistic symbioses. Because microbes found on a food are likely adapted to digesting this particular substance, it makes sense that an insect benefits from harnessing the talents of these microbes (Martin, 1992). The diversity of feeding modes, even over the ontogeny of an insect, supports symbioses among a diverse range of arthropods and microbes, and even different life stages of the same insect species may develop distinct symbioses (Jones, 1984).

15.2.1 Physiological Adaptations to Symbioses in Insects

There seems to be no end to the diversity of internal physiological structures where microbes of nutritional relevance to insects may reside, but close proximity to some portion of the digestive tract is commonly a prerequisite for these adaptations (Koch, 1960). The most obvious location of microbes of nutritional relevance to arthropods is within the gut lumen itself. Chapman (1998) points out that those insects with straight, simple digestive tracts have fewer nooks and crannies for symbiont communities to persist. Of the three main portions of the gut, the hindgut most frequently houses symbionts in insects (Bignell, 1984; Chapman, 1998; Koch, 1967). Within the digestive tract, microbial populations are often maintained and spatially restricted by the physiological conditions of the gut, especially pH (Bignell, 1984; Haas and Konig, 1988). For instance, in the detritivore, *Tipula abdominalis*, a narrow region of the midgut is maintained at a high pH (near 11). This is where Martin et al. (1980) believe symbiotic bacteria with strong proteolytic capabilities might reside and assist in the digestion of proteins bound to indigestible molecules (like tannins).

Any caecae, pockets, or structural anomalies of the gut may be specializations for housing symbiotic microbes (Koch, 1960). Chrysopid adults house yeasts within a large diverticulum that attaches to the posterior end of the foregut (Hagen and Tassan, 1966). Forbes (1892) provides a nice description of the diversity of caecae found in association with the midguts of various heteropterans, all of which house bacteria. Lygaeidae, Pentatomidae, Scutelleridae, Pyrrhocoridae, and Coreidae all have a series of gastric caecae that vary in their complexity and arrangement (see also Koch, 1967). In the cinch bug, between five and eight caecae radiate from a single point just before the end of the midgut. It is fascinating that even closely related genera within Heteroptera can vary dramatically in the arrangement (and even presence or absence) of these gastric caecae. Finally, the strongest associations between microbes and insects are manifested in specialized cells that house intracellular microorganisms, termed mycetocytes and mycetomes (Campbell, 1989; Chapman, 1998; Tanada and Kaya, 1993).

15.2.2 Nutritional Functions of Microbial Symbioses

Jones (1984) rightly highlighted the fact that microbes are seldom ubiquitous in their taxonomic and functional associations with arthropods. This is to say that no single microbe occurs widely in insects that remedies a taxonomically widespread nutritional deficiency. The functional outcome of each microbe-insect symbiosis needs to be considered on a case-by-case basis. Still, it is a useful exercise to briefly summarize the known contributions made by symbiotic microorganisms to their host's nutrition. The nutritional roles of symbiotic microbes can be typified as either contributing specific key nutrients required by arthropods, or enhancing or augmenting nutritional processes in the host. Studies on the contributions of microbes to insect nutrition should focus mainly on the nutrients that are consistently lacking from an insect's food source, but are critical to the fitness of the insect (Jones, 1984).

Symbiotic microorganisms can provide key nutrients to arthropods by serving as a food themselves, or by producing specific nutrients from substrates which insects cannot metabolize. In the former case, the microbes serve as the sole food source for the arthropod, or as a nutritional supplement to a suboptimal diet (Vega and Dowd, 2005; Jones, 1984). One example of this is observed in Diplopoda, in whom the yeast symbionts typically associated with Malpighian tubules venture into midgut, where they are digested (Byzov et al., 1993). More frequently reported are instances where the microbe provides some nutrient that arthropods cannot synthesize on their own. Most notable are B-vitamins (Akman et al., 2002; Campbell, 1989), sterols (Campbell, 1989; Morales-Ramos et al., 2000; Wetzel et al., 1992), and amino acids created from non-essential amino acid precursors (Campbell, 1989; Gil et al., 2003; Prosser and Douglas, 1991; Shigenobu et al., 2000).

In addition to the direct provision of nutrients to insect hosts, symbiotic microorganisms also augment normal digestive processes, allowing insects to make better use of what they eat. This result may arise from more efficiently extracting dilute nutrients from a food source, or providing the necessary enzymes to metabolize foods or byproducts more efficiently (Jones, 1984). Microbes digest molecules that many insects cannot, especially some of the structural and storage polysaccharides found in plant material (Martin, 1992). Specifically, microbes produce cellulases, pectinases, ligninases, and chitinases that enable arthropods to extract the most energy from their foods (Breznak and Brune, 1994; Campbell, 1989; Hogan et al., 1985; Howard et al., 1985; Hungate, 1938; Martin, 1984). In the omnivorous *Acheta domestica*, the hindgut microflora contributes a range of carbohydrases that broadens the suite of nutrients that can be extracted from low quality food

(Kaufman and Klug, 1991) (Fig. 15.2). Pectin, amylose, xylan, raffinose, and locust bean gum are all digested more efficiently in crickets with their symbionts intact versus in aposymbiotic conspecifics. When the quality of the dietary carbohydrates are deliberately changed periodically over nymphal development, the aposymbiotic crickets only grow when the high quality carbohydrates are offered; those with their symbionts intact grow even when times are nutritionally tough (Kaufman and Klug, 1991). It is worth noting that yeasts are unable to digest cellulose (Phaff et al., 1966), and therefore these symbionts do not fulfill this role in insects. In addition to providing digestive enzymes to arthropod hosts, microbes also contribute to the storage and recycling of nitrogen (Byzov et al., 1993; Campbell, 1989; Cochran, 1985; Douglas, 1998; Potrikus, 1981), sulfate assimilation (Douglas, 1998; Shigenobu et al., 2000), and fatty acid metabolism (Campbell, 1989 and references therein) in their hosts.

A final function of microbial symbionts is the detoxification of plant allelochemicals (and insecticides) harmful to the arthropod hosts (Vega and Dowd, 2005; Jones, 1984). In one early research system, a bacterial symbiont of *Rhagoletis pomonella*, *Pseudomonas melophthora* detoxifies six different insecticides under in vitro culture (Boush and Matsumura, 1967). Whether this happens in the host, or how the host accommodates the insecticide breakdown products is unknown. Still, the detoxification of plant secondary compounds (e.g., terpenoids) is commonly accomplished in insects by bacterial associates (Campbell, 1989 and references therein), and it is conceivable that these detoxification capabilities facilitate the degradation of pesticides in insects as well (Berenbaum, 1988). Of relevance to this book is the detoxification capabilities of microbe-symbiotic natural enemies that

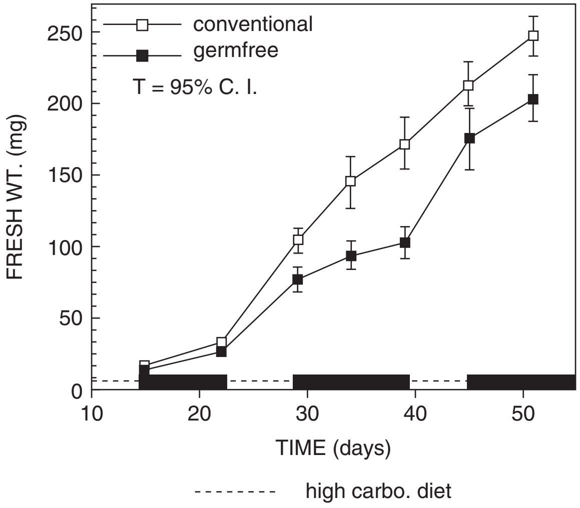

Fig. 15.2 Growth of aposymbiotic (germfree) and symbiotic (conventional) *Acheta domesticus* reared on an alternating diet regime with high and low quality foods (Reproduced from Kaufman and Klug., 1991. With permission by Elsevier)

allow them to consume chemically defended non-prey foods, but I know of no reports of this in the literature.

15.2.3 Natural Enemies and Microorganism Associations

> *Although there are many scattered reports on the bacteria associated with insects, knowledge concerning the bacterial flora of insects in general is markedly scant. Since there are over 600,000 known species of insects, it is apparent that a considerable amount of work will be necessary before even a limited survey of this field can be accomplished. In the meantime it seems logical that a study of a few representative species of Hexapoda might be worthwhile.* (Steinhaus, 1941)

15.2.3.1 Chrysopidae

Since first discovered 85 years ago (Cowdry, 1923), and popularized nearly 50 years ago (Hagen and Tassan, 1966), the microorganisms (especially yeasts) associated with green lacewings have been the focus of a considerable body of research that provided an excellent example of the microbial promotion of dietary breadth in arthropods best appreciated for their entomophagous tendencies. In spite of a wealth of information that has been acquired regarding the symbiotic relationships between these two sets of organisms, the microbial contributions to the nutrition of lacewings has not yet been empirically substantiated.

Ken Hagen and colleagues prompted a long-lived line of research that continues to this day with the following statement:

> *Budding yeasts were found in the diverticulum of the adult foregut of* [Chrysoperla] carnea. *The yeast was found in both the laboratory stock as well as field collected specimens. It is speculated that the yeast may play a mutualistic role in the synthesis of essential metabolites in the host which are often lacking in the natural adult diet, honeydew.* (Hagen and Tassan, 1966)

Adult lacewings was shown to possess a large diverticulum that joined the digestive tract directly anterior to the midgut (Fig. 15.3). This diverticulum was often filled with yeasts, initially assigned to the genus *Torulopsis* (Hagen and Tassan, 1972; Hagen et al., 1970). Investigations of larval guts did not produce yeasts, and Hagen et al. concluded that the relationship between lacewings and yeasts was a loose one, perpetuated when adults consumed the yeasts in contaminated honeydew or nectar which were then passed among a lacewing community through trophalaxis. Perhaps even more interesting, female lacewings were shown to possess tracheae of broader diameter that presumably provided additional oxygen to the symbiotic yeasts (Hagen et al., 1970).

A number of recent explorations validate that lacewings are frequently host to a diverse community of microorganisms, many of which are yeasts in the genera *Candida* and *Metschnikowia* (= *Torulopsis*, in part) (Chen et al., 2006; Nguyen et al., 2006; Suh et al., 2004; Woolfolk and Inglis, 2004). Although Hagen et al. (1970) suggest that European and North American *Chrysoperla carnea* have different

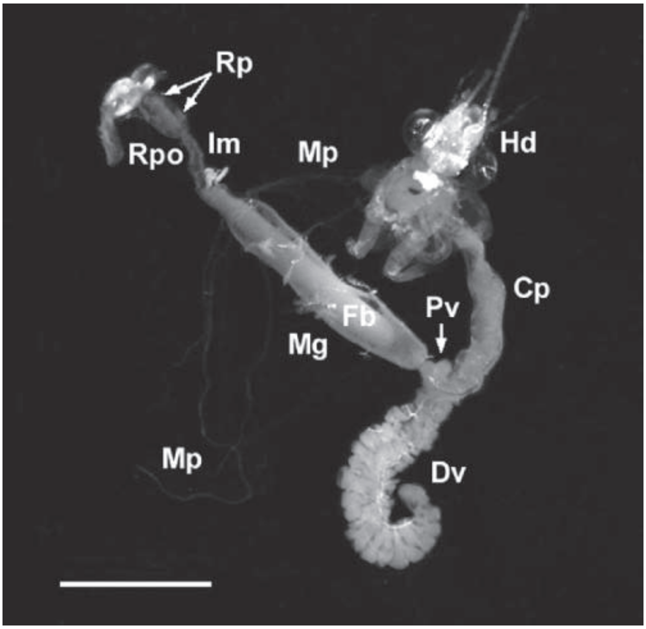

Fig. 15.3 Alimentary canal of an adult *Chrysoperla rufilabris* where symbiotic yeasts reside. Cp, crop; Dv, diverticulum; Fb, food bolus; Hd, head; Im, ileum; MG, midgut; Mp, Malpighian tubules; Pv, proventriculus; Rp, rectal pads; Rpo, rectal pouch (Reproduced from Woolfolk et al., 2004. With permission from the Entomological Society of America)

yeast symbionts, more recent descriptions reveal a fairly strong fidelity between lacewings and certain yeast species (Suh et al., 2004). Specifically, the same yeasts *Candida pimensis* and *C. picachoensis*, are found in the guts of lacewings collected in geographically distant populations (Arizona and Louisiana), suggesting that either these yeasts are extremely widespread, or that there is a strong relationship between yeast and host (Nguyen et al., 2006). *Metschnikowia pulcherrima* are found in the guts of all *Chrysoperla rufilabris* that have yeast symbionts (71% of individuals), with cell counts on the order of 5×10^2–10^5 (Woolfolk and Inglis, 2004). Transient filamentous fungi and bacteria are also found in the guts of this lacewing species. Recently eclosed lacewings do not possess yeast symbionts (Woolfolk and Inglis, 2004), and likely have to acquire them from the environment. Not found in Hagen's early work is that the larvae of *Chrysoperla carnea* also host a diverse community of microorganisms, but few yeasts (Chen et al., 2006). Chen et al. suggest that because the larval midgut is closed at the junction to the hindgut, the dense populations of free-living bacteria in the midgut lumen may be important in maximizing the digestion of the food material. Finally, Gibson and Hunter (2005) describe how *Chrysoperla* mothers transfer yeasts to their egg surfaces, but lack of yeasts in the larvae of lacewings and newly eclosed adults seems to preclude vertical maintenance of the symbiosis.

Several physiological adaptations promote the symbioses between yeasts and lacewings. Canard and colleagues (Canard, 2001; Canard et al., 1990) expound upon the initial observation of Hagen et al. (1970) that yeast-bearing *Chrysoperla carnea* have increased tracheation to the diverticulum. Specifically, Canard et al. (1990) provide data that clearly show that glucophagous lacewing adults have tracheae of greater diameter near the diverticulum compared to predaceous lacewing species. These morphological adaptations to symbiosis with yeasts suggest a strong physiological benefit to the lacewing. Within the digestive system of the adults, the yeasts are restricted to the foregut and diverticulum (Woolfolk and Inglis, 2004), but some make their way into the midgut lumen and hindgut (Chen et al., 2006; Woolfolk and Inglis, 2004). This residency pattern may be reinforced by the presence of forward-pointing hairs that line the proventriculus of *Chrysoperla* (Woolfolk et al., 2004) (Fig. 15.4). Little absorption occurs within the diverticulum itself, which is lined with cuticle (Woolfolk et al., 2004).

The repeated demonstration of diverse yeasts in the guts of lacewings, and the morphological adaptations to housing the yeasts in the lacewing diverticulum suggest that these yeasts are providing some beneficial function to the lacewing. The honeydew diet of chrysopids with the strongest yeast associations does not contain the requisite nutrients for maximum oogenesis (Hagen and Tassan, 1972). Honeydews have minimal amino acid contents, and feeding trials suggest that the yeast symbionts may be supplementing the diets of glucophagous lacewings with the key amino acids, valine, threonine, and phenylalanine (Hagen and Tassan, 1972; Hagen et al., 1970). These authors also point out that the yeasts do not improve oviposition rates when fed certain honeydews, and recognize that the interactions between yeasts and lacewings may be more complex. Many of Hagen et al.'s assertions regarding the function of yeasts in lacewings are recently challenged by Gibson and Hunter (2005), largely based on the inability to replicate the results of these early experiments. First, Hagen et al. use sorbic acid to cure their lacewings, which does not produce aposymbiotic individuals in Gibson and Hunter's experiments. Gibson and Hunter produce aposymbiotic lacewings using cycloheximide, but they don't

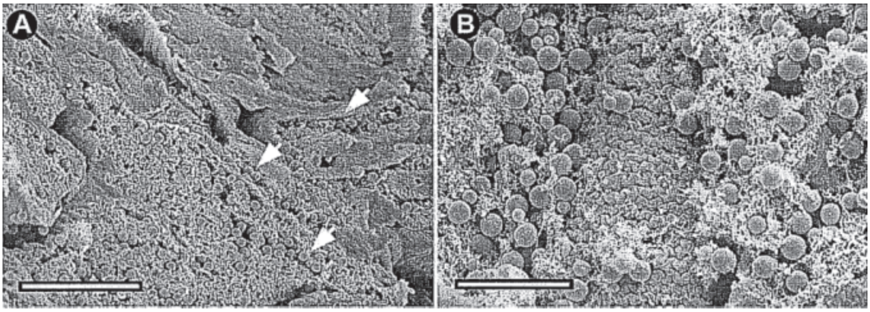

Fig. 15.4 Scanning electron micrographs of *Chrysoperla rufilabris* adult foregut with high populations of yeast (arrows) in between fold structures within cryofractured foregut. Bar 50 µm (Reproduced from Woolfolk et al., 2004. With permission from the Entomological Society of America)

use this treatment to explore the nutritional contributions of the symbionts. The latter work shows that the number of yeasts is significantly correlated with total fecundity in the females, but the authors challenge the notion that this improvement is based on amino acid content in the diet. In replicating Hagen et al.'s amino acid supplementation experiments, a consistent trend in Gibson and Hunter's work is that inclusion of the amino acid valine seems to actually reduce both yeast count and realized fecundity in the lacewings. The idea that lacewings consume honeydew and nectar in part to feed their yeast symbionts, which then form part of the diet in the lacewing is suggested by Woolfolk et al. (2004) and Chen et al. (2006), but no empirical studies test this hypothesis. The bottom line is that the contribution of yeast symbionts to the nutrition of lacewings remains to be imperviously established.

15.2.3.2 Crickets

Crickets are one of the 'lab rats' used to understand the contributions of symbiotic microorganisms to insect nutrition. A number of species have bacterial residents in their guts (Kaufman et al., 1989; Steinhaus, 1941), and these symbionts are maternally inherited in some species (Koch, 1960). In *Acheta domesticus*, microorganisms residing in the hindgut enhance the diversity of polysaccharides that can be consumed by the insect (Kaufman et al., 1989). These microorganisms allow the cricket to adapt to a changing diet more quickly, thereby speeding development and improving fecundity over aposymbiotic crickets when both groups are faced with a suboptimal diet, or a suboptimal diet periodically alternated with a more digestible one. Gryllids possess the same carbohydrase profile in their hindgut as *A. domesticus*, suggesting a similar symbiosis with microorganisms (Kaufman and Klug, 1991). Of reference to granivory, removal of microbial gut populations with antibiotics is associated with a 40% reduction in seed (*Chenopodium album*) consumption by *A. domesticus* (J. G. Lundgren , unpublished data 2006).

15.2.3.3 Heteroptera

A number of heteropterans have symbiotic microorganisms that facilitate herbivory, and although there are numerous reports on the most entomophagous heteropterans hosting a diverse gut community, the functions of these symbionts remain a mystery. Forbes (1892) is one of the first to document specialized organs (gastric caecae) that house bacteria in several families of Heteroptera, but especially in the cinch bug. He even notes that the caecae of the Coreidae have increased tracheation, similar to the diverticula of lacewings described above. After identifying the bacterial associate of the chinch bug as *Micrococcus insectorum*, he basically ceases this line of research.

> *I have no present desire to speculate concerning the meaning of the bacterial contents of these glands, but limit myself to this preliminary account…* (Forbes, 1892)

Glascow reveals some of Forbes' later unpublished findings, summarizing them as

> [Forbes] *also established later, by the examination of a great variety of insects of different orders, and especially of Heteroptera, that the chinch bug was not unique in this regard, but that the same phenomenon also occurred in a number of other species of Lygaeidae as well as in representatives of several other families of Heteroptera, and that wherever the caeca were present in this group, they were always filled with specific bacteria.* (Glascow, 1914)

Glascow advances these initial observations by determining that microorganism species vary among hosts, but are fairly consistent within a host species. Moreover, he found that the bacteria, which cannot be cultured, can be maternally inherited by offspring. A number of other researchers have since frequently found microbial associates of herbivorous heteropterans (Haas and Konig, 1988; Hosokawa et al., 2007; Kikuchi et al., 2007; Koch, 1967; Martin et al., 1987; Prado et al., 2006; Ragsdale et al., 1979), and blood-sucking triatomine reduviids (Buchner, 1965; Koch, 1967).

The symbiotic microbes of entomophagous species inarguably receive less attention from biologists, although there are clues that point to dynamic relationships worth considering in these natural enemies. Of the most predaceous species, Nabidae, Reduviidae, *Geocoris uliginosus* and *Podisus maculiventris* do not have the caecae necessary for harboring bacterial symbionts (Forbes, 1892; Glascow, 1914). This doesn't preclude them from having nutritional symbionts. Indeed, Glascow describes the same species of bacteria in the guts of *P. maculiventris* (present in 50% of individuals) as is seen in the caecae of the herbivore *Holcostethus limbolarius*. And Cowdry (1923) describes fungi and bacteria living in the guts of a *Nabis* species. Other predaceous heteropterans have at least transient bacterial residents in their guts as well; *Sinea diadema* (Reduviidae) and *Lygus pratensis* (a facultatively entomophagous Lygaeidae) both have two bacterial species residing in their guts (Steinhaus, 1941). Also, gerrids have a number of gut symbionts, which do not exceed 36% infection of surveyed insects and are not pathogenic to the bugs (Klingenberg et al., 1997). Still, the absence of caecae does suggest that the relationships are weaker in entomophagous species than they are in herbivorous and blood-sucking heteropterans, and the contribution of these resident microorganisms to the nutrition of entomophagous insects is entirely unknown.

15.2.3.4 Coleoptera

With the recent exception of the Carabidae, very little is known of the microbial residents of predatory beetles. One report states that flower-visiting cantharid adults (*Raxonycha* species) have yeasts (*Metschnikowia corniflorae*) in their guts (Nguyen et al., 2006), and another species has fungi in its gut (Cowdry, 1923). A number of unidentified yeasts were isolated from the stomachs of basidiocarp-dwelling carabids and staphylinids (Suh and Blackwell, 2005). Buchner (1965) discusses how mycetomes are present in the Malpighian tubules of pollinivorous *Dasytes* females, but not the males. In his broad survey for microbes in the digestive tracts of insects, Steinhaus (1941) determines that the lampyrid, *Photinus pyralis*, *Coccinella novemnotata*, and a coccinellid larva have bacteria in their guts. *Adalia* *bipunctata* is known to have a *Rickettsia* inhabitant in its intestinal lumen (Cowdry, 1923). Although Forbes (1892) mentions that carabid beetles have gastric caecae similar to the Heteroptera, no bacteria are associated with these organs according to his notes. Cowdry (1923) describes the presence of bacteria in the gut lumen of *Cicindela punctulata punctulata* and numerous *Rickettsia* within the digestive tract of *Anisodactylus agricola*.

Recent explorations by myself and colleagues reveal that carabids have a rich bacterial community associated with their digestive tracts, and one function of these bacteria may be in facilitating seed consumption by facultatively granivorous species. Direct cell counts of the bacterial community in the guts of field-collected (and primarily predatory) *Poecilus chalcites* reveal 1.5×10^8 bacteria ml^{-1} of gut (Lehman et al., in press), and reducing the dietary breadth of the insects through lab culture reduces the bacterial diversity within this species. In another study, the guts of two granivorous carabids, *Harpalus pensylvanicus* and *Anisodactylus sanctaecrucis*, both harbor simple yet consistent bacterial communities that are distinct between the two carabid species (Lundgren et al., 2007). Perhaps what is more interesting is that curing these two species of their bacterial associates with antibiotics reduces the consumption of *Chenopodium album* seeds by 40% (Lundgren et al., 2007). The exact contributions of these gastric bacteria to digestion of seed material remain to be established.

15.2.3.5 Formicidae

Blochmann's early discovery that *Camponotus ligniperdus afer* and *Formica fusca fusca* harbor endosymbionts was the first report of an insect-microbe mutualistic symbiosis (Buchner, 1965; Koch, 1960) (note that another *Camponotus* also has bacterial gut residents; Cowdry, 1923). Based on embryonic development, Koch (1967) believed that congeners *F. rufa rufa* and *F. sanguinea* may have had similar bacterial symbioses to those of *F. fusca fusca* that have secondarily been lost. More recent work shows that nutritional symbiosis between microbes and omnivorous ants may be much more pervasive and integral than early workers could have imagined. In *Camponotus floridanus*, bacterial symbionts in the genus *Blochmannia* (especially *floridanus*) provide a series of amino acids to the workers, most notably tyrosine and the essential amino acids phenylalanine and methionine (Feldhaar et al., 2007; Zientz et al., 2006). Also important, these bacteria aid in nitrogen metabolism and reducing sulfate to sulfide for their host (Feldhaar et al., 2007; Zientz et al., 2006). Aposymbiotic workers that are amino-acid limited produce fewer brood than symbiotic workers and aposymbiotic workers supplemented with a dietary source of amino acids (Feldhaar et al., 2007). These bacteria are intracellular, living within specialized baceteriocytes housed in the midgut epithelium and in the ovaries, and they are likely transmitted vertically to the brood (Wolschin et al., 2004). Several other genera within the Formicinae are known to harbor this bacterium, and Feldhaar et al. (2007) speculate that this association has persisted for 30–40 million years. Indeed, these symbiotic interactions may have facilitated the current species diversity present in this genus.

Other ants also harbor gut bacteria that may contribute to their nutrition. *Tetraponera* species have a number of bacterial associates in their guts (Stoll et al., 2007), as do *Solenopsis* colonies. In an elegant study pertaining to nutritional symbionts of fire ants, Ba and Phillips (1996) determine that colonies of *Solenopsis invicta* in the southern U. S. are associated with several *Candida* species and *Debaryomyces nasenii* var. *hansenii*. The most abundant microbes (90% of the microbial community) are *Candida parapsilosis* and *C. lipolytica*, and these species are geographically widespread and prevalent throughout the season. Very few of the adults (3.27%) and third instars harbor the yeasts, but 80% of fourth instars in 100% of the colonies are symbiotic, mostly with *C. parapsilosis*. In that this life stage is where most of the digestion of solid food material occurs within the colony, it seems likely that the yeast is providing some nutritional function. Ba and Phillips (1996) discuss evidence that points toward the yeasts' contribution of the sterols, ergosterol and zymosterol, to the nutrition of the colony.

15.2.3.6 Other Natural Enemies

A handful of explorations describe the microbiota of the digestive tracts of a range of other natural enemies. Yeast-like intracellular symbionts are apparently transferred vertically to the progeny of the ichneumonid *Pimpla turionellae* (Middledorf and Ruthmann, 1984). These yeasts are found in the hemolymph and fat body of the adult wasp, and may contribute to the wasp's nutrition through the frequent passage of large vacuoles across the cell membrane. For the most part, the function of yeasts associated with parasitoids is largely unknown (Vega and Dowd, 2005). The ichneumonids, *Casinaria infesta* and *Echthromorpha maculipennis*, also are associated with bacteria (Cowdry, 1923). Predatory wasps also have bacterial associates (Cowdry, 1923). Of the entomophagous Diptera, two out of four syrphid adults (*Eristalis* species) tested have bacteria in their guts (Cowdry, 1923).

The Arachnida also have known bacterial associates. *Enterobacter* was isolated from *Galendromus occidentalis* (Hoy and Jeyaprakash, 2005), and the bacterial symbiont *Aranicola proteolyticus* (= *Serratia proteamaculans*) contributes the broad-spectrum protease, arazyme, that may be useful in the digestive processes of the spider *Nephila clavata* (Bersanetti et al., 2005). The jumping spider, *Salticus scenicus*, may have maternally inherited microbes associated within its haemocoel (Cowdry, 1923). A crab spider, *Misumena vatia*, has many protozoa-like microbes in its gut, and *Leiobunum vittatum dorsatum* has intestinal bacteria as well (Cowdry, 1923). Also, Cowdry found some microbes in the guts of the centipedes *Scolopendra subspinipes subspinipes*, and *Scutigera forceps*. Beyond these superficial descriptions, little is known concerning the functions of these relationships between other natural enemies and their gut microbes.

15.3 Conclusions

While there are numerous descriptions of the microbial diversity symbiotic with non-prey foods or the digestive systems of entomophagous species, we are only just beginning to understand the importance of these interactions. Nevertheless, some putative ecological functions and processes are assigned to microbes (alterations in the palatability of non-prey foods to natural enemies, and increasing the dietary breadth of entomophagous species) but their extent needs to be further resolved. It is clear that microorganisms play an important part in the lives of natural enemies, and how symbiotic interactions drive the evolution of omnivory in entomophagous species and their relationships with non-prey foods will likely prove to be a fruitful branch of research in the near future.