

# EXTRA AND INTRACELLULAR MICROORGANISM AND THEIR RILE IN INSECT PHYSIOLOGY

## 1. ECTO-SYMBIOTIC FUNGI

Several insects have ectosymbiotic relationships with fungi. The insects eat the fungus, but the association differs from that in most fungus-eating insects, because the insect manipulates the fungus, and so derives nutrients, indirectly, from substrates that would otherwise be difficult or impossible for it to utilize. Ambrosia beetles (some Scolytinae and nearly all Platypodidae) are associated with fungi that enable them to use the xylem of woody plants. The fungi are the principal food of both larvae and adults, and their key role is probably in concentrating nitrogen, present in very low concentrations in the wood. They also provide sterols, such as ergosterol, which are essential for the beetles' development. Bark beetles (most Scolytinae) feed largely on the phloem of woody tissues which is higher in nutrients than the xylem. They also have fungal associations, but their dependence is less extreme. The beetle–fungus associations are not species-specific. Several fungus genera are associated with ambrosia beetles. Two of the best known are *Fusarium* and *Ambrosiella*. Most of those associated with bark beetles are in the genus *Ceratocystis*. Leaf-cutting ants (Attini) are dependent on specific fungi for larval food. Worker ants cut leaves, and other parts, from living plants and carry them to the nest. Here, the ants chew the plant fragments, removing the waxy cuticle and possibly also removing existing micro-organisms on the plant surface. Using faeces, they build the chewed fragments into a garden which they inoculate with hyphae from an existing garden. The fungi are *Basidiomycetes* that only occur in the nests of these ants. Macrotermitinae also cultivates fungi in gardens, called fungus combs, constructed from fresh faecal material containing wood fragments. The fungus, in the genus *Termitomyces*, is only found associated with termites. It breaks down cellulose and lignin and, when ingested by the termites, it contributes its cellulolytic enzymes to those of the insect. Nitrogen is also concentrated. In reproductive structures of the fungus, which are eaten by the termites, it reaches 8% dry weight; the wood initially ingested may have only about 0.3% dry weight. Termitidae, including the Macrotermitinae, do not have endosymbiotic protozoa, unlike all the other termites.

## 2. ENDOSYMBIONTS

Many insects have micro-organisms extracellularly in the gut lumen or intracellularly in various tissues.

### **Micro-organisms in the alimentary canal**

Micro-organisms are almost inevitably ingested during feeding and so an intestinal flora is present in most insects. The alimentary canal of grasshoppers, for instance, is sterile when the insect hatches from the egg, but soon acquires a bacterial flora which increases in numbers and species throughout life. In general, insects with

straight alimentary canals contain fewer micro-organisms than those with complicated guts which have a range of different pH values, providing many different niches. The micro-organisms occurring in the gut in these cases of casual infection largely reflect what is present in the environment. At least in the case of the locust, *Schistocerca*, and larval fruit flies, *Rhagoletis*, the gut flora does not contribute to the nutrition of the insect. Adult tephritid flies have a dorsal diverticulum of the oesophagus in which bacteria accumulate. In general, the species of bacteria present reflect what is present on the surface of host fruit, although some species do occur consistently and in greater abundance than others. It is not known if these bacteria are true symbionts, or if they contribute to the nutrition of the flies. In other cases, it is known that micro-organisms in the gut contribute to the insect's nutrition. Detritus-feeding cockroaches, such as *Periplaneta*, and crickets have bacteria in the hindgut. They may be attached to projections from the intima of the hindgut and they enhance the insects' ability to digest plant polysaccharides, such as xylans, pectins and gums, and oligosaccharides, such as raffinose. Crickets do not have enzymes capable of digesting these compounds. The bacteria produce short-chain fatty acids which are absorbed in the hindgut. These associations involve a variety of bacterial species. Whether or not some are characteristic is not known.

Scarab beetle larvae (Scarabaeidae), which feed on decaying wood, have a bacterial flora housed in an expansion of the hindgut. It is believed that the bacteria are those commonly involved in the process of wood decay and that they are ingested when the wood is eaten. In this case, the association is not truly symbiotic, although the larvae are dependent on the bacteria for digesting the food. Some termites and wood-eating cockroaches have flagellates in the hindgut which are important in the digestion of wood. These insects have a strict symbiotic relationship with their hindgut fauna, and the behaviour of the insects ensures transfer from generation to generation. The bacteria which are present in the guts of higher termites can fix atmospheric nitrogen which is subsequently incorporated into the tissues of the insects.

**Table 1: Insects feeding on nutritionally poor diets and their associated symbiotic organisms**

Type of food	Insect order/family	Micro-organisms		Contribution to insect
		Position in body	Type	
Wood	<b>Blattodea</b>			
	<i>Cryptocercus</i>	Hindgut	Flagellates	Cellulose digestion
	<b>Isoptera</b>			
	Kalotermitidae	Hindgut Hindgut	Flagellates Bacteria	Cellulose digestion
	Macrotermitinae	Ectosymbionts	Fungi	Cellulose digestion Concentration of nitrogen
	<b>Coleoptera</b>			
	Anobiidae	Midgut cecal epithelium	Yeasts	Essential amino acids
	Scolytinae	Ectosymbionts	Fungus	Concentration of nitrogen
Platypodidae	Ectosymbionts	Fungus	The concentration of nitrogen and sterols	

		Hindgut	Bacteria	Nitrogen fixation
	<b>Hymenoptera</b>			
	Siricidae	Ectosymbionts	Fungus	Cellulose digestion
Green plants	Attini	Ectosymbionts	Fungus	Cellulose digestion
Phloem	<b>Homoptera</b>			
	Aphididae	Hemocoel	Bacteria	Amino acids
	Delphacidae	Hemocoel	Bacteria, yeast	Amino acids, sterols
Vertebrate blood	<b>Phthiraptera (Mallophaga)</b>	Variable	Bacteria	B vitamins
	<b>Anoplura (Siphonculata)</b>			
	<b>Hemiptera</b>			
	Cimicidae	Hemocoel	Bacteria	B vitamins
	<b>Diptera</b>			
	Glossinidae	Midgut epithelium	Bacteria	B vitamins
Detritus	<b>Blattodea</b>	Hindgut	Bacteria	Carbohydrate digestion
		Fat body	Bacteria	Nitrogen recycling

### Intracellular micro-organisms

Intracellular micro-organisms fall into *two groups*: those that occur in otherwise normal cells of an insect, and those that are restricted to special cells with discrete morphology known as **mycetocytes**. The former has been recorded from many insect orders. In general, they appear to have no effects on the biology of the host insect. However, this is not true of the bacterium *Wolbachia*, which is known to be present in ovarian tissue of some species of Orthoptera, Hemiptera, Coleoptera, Diptera, Hymenoptera and Lepidoptera. It is transmitted cytoplasmically and causes post-zygotic incompatibility between different strains of various species, including *Tribolium confusum* and *Culex pipiens* and to cause parthenogenesis in some parasitic Hymenoptera, such as *Trichogramma*.

### Mycetocyte micro-organisms

Mycetocyte micro-organisms are universal amongst species that feed on vertebrate blood throughout their lives: Cimicidae (bedbugs), Triatominae (kissing bugs), Anoplura (sucking lice), Glossinidae (tsetse flies), Hippoboscidae (deer flies) and Nycteribiidae (bat flies), but they are not found in fleas, mosquitoes or horseflies that have larvae which are not blood-sucking. They are almost universal amongst Homoptera, the only exceptions being those feeding on tissues other than the phloem or xylem. Many, but not all, wood-feeding beetles and *Ischnocera* (Mallophaga), feeding on feathers and skin debris of birds, also have mycetocyte micro-organisms. Micro-organisms in mycetocytes are also found in all cockroaches in the family Blattidae and ants of the tribe Camponoti. These are omnivorous insects, but it is suggested that their diet is often poor with an imbalance of amino acids.

### Types of micro-organism

The mycetocyte micro-organisms of aphids and weevils are bacteria and this is probably also true of the mycetocyte micro-organisms in a majority of other insects.

Yeasts are present in Fulgoridae and *Laodelphax* amongst the Homoptera and Anobiidae and Cerambycidae amongst wood-boring beetles. In Triatominae the microorganisms are Actinomycetes. In general, only one form of micro-organism is found in each insect species, but this is not true in many Homoptera. All Auchenorrhyncha appear to house more than one type of micro-organism and, in Fulgoridae, both yeasts and bacteria are present. Some species have as many as six different symbionts. Many aphid species have only a single bacterial symbiont, but others may have two or three different types; some also have yeasts.

### Location in the insect body

Mycetocytes are large polyploid cells. They are scattered amongst the principal cells of the midgut epithelium in *Haematopinus* (Anoplura), while in cockroaches they are scattered through the fat body. In other insects, the mycetocytes are aggregated to form organs known as mycetomes, often in the hemocoel. Amongst holometabolous insects and some Homoptera, a mycetome is often only present in the larval stages. At metamorphosis, it fragments into mycetocytes which become lodged in adult organs. The larval mycetome of the beetle *Calandra* is a U-shaped structure below the foregut, but not connected to it; in the adult, mycetomes are present in the epithelium of the midgut ceca. In Hippoboscidae and Glossinidae, the mycetome is present as a discrete zone in the midgut epithelium. Mycetocytes generally do not divide; they increase in size, and endomitotic divisions lead to polyploidy. During the larval and early adult period of aphids, individual cells may increase in volume about 10-fold in apterous (wingless) morphs, but to a lesser extent in alates (winged forms). The biomass of symbionts increases in parallel with the volume of the cells (Fig. 4.15). In alates, the number of mycetocytes declines sharply when the insects become adult, but, in apterous individuals, this sharp decline is delayed (Douglas & Dixon, 1987). It is not known how these changes are regulated. Within the mycetocytes of most insects, each microorganism is in a separate vacuole surrounded by a membrane, but in *Glossina* and ants, the symbionts are free in the cytoplasm.

**Table 2: Survey of mycetocyte symbioses in insects**

Insects	Microorganisms
<b>Homoptera</b>	
Auchenorrhyncha (including leafhoppers, planthoppers, cicadas)	<i>Sulcia muelleri</i> (Bacteroidetes); and <i>Baumannia cicadellincola</i> ( $\gamma$ -proteobacteria) in Cicadellinae, <i>Zinderia insecticola</i> ( $\beta$ -proteobacteria) in Cercopidae, <i>Hodgkinia cicadicola</i> ( $\alpha$ -proteobacteria) in Cicadae and Pyrenomycete fungi in some Fulgoridae
Aphids	<i>Buchnera aphidicola</i> ( $\gamma$ -proteobacteria) or pyrenomycete fungi
Whitefly	<i>Portiera aleyrodidarum</i> ( $\gamma$ -proteobacteria)
Psyllids (jumping lice)	<i>Carsonella ruddii</i> ( $\gamma$ -proteobacteria)
Scale insects & mealybugs	<i>Tremblaya principis</i> ( $\beta$ -proteobacteria), <i>Moranella endobia</i> ( $\gamma$ -proteobacteria)
<b>Heteroptera</b>	

Cimicids (bed bugs)	Wolbachia ( $\alpha$ -proteobacteria) in <i>Cimex lectularius</i>
Triatomine bugs	<i>Arsenophonus triatominarum</i> ( $\gamma$ -proteobacteria)
<b>Anoplura (Siphunculata)</b> (sucking lice)	<i>Riesia pediculicola</i> ( $\gamma$ -proteobacteria) in human head louse & body louse
<b>Diptera</b> Pupiparia	<i>Wigglesworthia</i> ( $\gamma$ -proteobacteria) in tsetse flies
<b>Blattidae</b> (cockroaches)	<i>Blattabacterium</i> (flavobacteria)
<b>Mallophaga</b> (biting lice)	Not known
<b>Psocoptera</b> (booklice)	Rickettsia ( $\alpha$ -proteobacteria)
<b>Beetles, weevils and Anobiid timber beetles</b>	Various $\gamma$ -proteobacteria (including <i>Nardonella</i> ) <i>Symbiotaphrina</i> (yeasts)
<b>Hymenoptera, Camponoti</b> (carpenter ants)	<i>Blochmannia</i> ( $\gamma$ -proteobacteria)

### 3. CONTRIBUTION OF SYMBIOTIC MICROORGANISMS TO INSECT NUTRITION

An estimated 10% of all insects utilize diets that are nutritionally so poor or unbalanced that they depend on resident, beneficial microorganisms for sustained growth and reproduction. In some insects, the microorganisms degrade complex dietary components to a form that can be assimilated by the insect. The cellulose-degrading microbiota, that supplements the intrinsic cellulase enzymes in lower termites and some other insects. Other microorganisms have a biosynthetic function, and they are considered here. The chief nutrients that insects derive from microorganisms are essential amino acids (especially in insects feeding on plant sap), vitamins (insects feeding on vertebrate blood through the lifecycle) and sterols (for example in various insects utilizing wood). The role of microorganisms in essential amino acid synthesis is one aspect of various ways in which microorganisms provide solutions to a widespread nutritional problem for insects – nitrogen hunger, as is considered first.

#### Symbiotic microorganisms and provision of nitrogen

Symbiotic microorganisms promote insect utilization of low-nitrogen foods in various ways. One way is to concentrate the nitrogen. For example, the fungi cultivated by Macrotermitinae produce nitrogen-rich nodules on which the termites feed. Other ways involve metabolic capabilities of the microorganisms that are absent from the insect, including the utilization of insect nitrogenous waste compounds (e.g., uric acid), synthesis of “high-value” nitrogenous compounds (e.g., essential amino acids) and nitrogen fixation. Microbial utilization of insect nitrogenous waste products conserves nitrogen, in that the microorganisms utilize nitrogenous compounds that would otherwise be excreted, so reducing their use of nitrogenous compounds valuable to the insect host. This interaction has been demonstrated for bacteria, including *Bacteroides* and *Citrobacter* species in the hindgut of termites such as *Reticulitermes flavipes*, and clavicipitacean fungi associated with the haemolymph and fat body of some plant hoppers, notably *Nilaparvata lugens*. In termites, the nitrogen is recycled, *i.e.*, the bacteria metabolize

nitrogen from the insect-derived uric acid to amino acids, which are transferred back to the termite and incorporated into protein. In this way, microbial nitrogen recycling transforms waste nitrogen to high-value nitrogenous compounds, including essential amino acids of high nutritional value to the insect host. Cockroaches do not excrete uric acid; they store it in cells in the fat body. These stores become depleted if the insect feeds on a diet low in nitrogen. The symbionts can synthesize a range of essential amino acids, including those containing sulphur. Essential amino acid synthesis is also important for insects feeding on plant sap through the lifecycle. Plant-sap feeding has evolved multiple times in the Hemiptera, but apparently in no other insects, and it is invariably associated with the possession of microorganisms. In many hemipterans, the microorganisms are restricted to a particular cell type called the mycetocyte (also known as *bacteriocyte*). Nevertheless, some plant-sap feeding hemipterans bear microorganisms in different locations (e.g., in the posterior midgut of pentatomid stink bugs), and the mycetocyte symbioses are not restricted to hemipterans, but occur in several different insect orders. In all mycetocyte symbioses of insects, the microorganisms are transmitted vertically from mother to offspring, usually via the egg, and both insect and microbial partners require the association. The location of the mycetocytes is, however, very variable and includes the gut, hemocoel and fat body. The possession of symbiotic microorganisms by plant-sap feeding hemipterans has been correlated and attributed to the low essential amino acid content of phloem and xylem sap. Direct evidence that symbiotic microorganisms provide essential amino acids has been obtained for aphids, most of which bear the  $\gamma$ -proteobacterium *Buchnera aphidicola* in mycetocytes in the hemocoel. When aphids are reared on diets from which individual essential amino acids are omitted, the growth of aphids containing their normal complement of *Buchnera* is not affected, but aphids experimentally deprived of *Buchnera* by antibiotic treatment grow very poorly. In support of these dietary data, radiotracer experiments have confirmed that *Buchnera* synthesizes essential amino acids from radioactively labelled precursors, and release these labelled essential amino acids to the aphid tissues. These physiological data have been confirmed spectacularly by genome sequencing analyses showing that *Buchnera* has the genetic capacity to synthesize essential amino acids, despite its small genome size (0.6 Mb, less than 20% of the size of the genome of the related bacterium *Escherichia coli*). *Buchnera* may also provide the insect with riboflavin. The taxonomic identity of symbiotic microorganisms varies among different groups of plant-sap feeding hemipteran insects, and all are believed to provide essential amino acids, although this has not been demonstrated by physiological methods. The genomic approach has been applied to investigate the nutritional role of symbiotic bacteria in a xylem-feeding hemipteran, the auchenorrhynchan *Homalodisca coagulata* (the glassy-winged sharpshooter). As with various other auchenorrhynchan groups, this insect bears two bacteria: *Baumannia cicadellincola*, a  $\gamma$ -proteobacterium, and *Sulcia muelleri*, a member of the Bacteroidetes. These bacteria have very small genomes and restricted but complementary predicted metabolic capabilities. *Sulcia* can synthesize essential amino acids and *Baumannia* can synthesize various vitamins and cofactors.

Furthermore, the putative capabilities of the two bacteria are interdependent. For example, *Sulcia* synthesizes **homoserine**, the substrate for the synthesis of the essential amino acid methionine by *Baumannia*, and *Baumannia* provides the polyisoprenoids required for menaquinone synthesis by *Sulcia*. In other words, this insect is dependent on cross-feeding of metabolites between the two bacteria. A final, potentially important way for insects to gain nitrogen is by association with bacteria that can fix atmospheric nitrogen. Nitrogen-fixing bacteria have been demonstrated in a few insects, including a minority of termites, the wood-feeding larvae of the stag beetle, *Dorcus rectus*, and in fruit flies, particularly the medfly, *Ceratitis capitata*, which contains large populations of nitrogen-fixing enterobacteria in its guts. Rather little research, however, has been conducted on the nutritional significance of nitrogen-fixing bacteria to insects.

### **Microbial production of vitamins**

Some of the best evidence for microbial provisioning of vitamins comes from research on insects feeding on vertebrate blood through the lifecycle, including the tsetse flies, *Glossina*, other Diptera Pupiparia, the anopluran “sucking” lice and the Cimicids (bed bugs). Vertebrate blood is notoriously deficient in B vitamins, and the microbial symbionts in these insects, all of which are localized to mycetocytes have been implicated in B vitamin provisioning. Insects that utilize blood solely as adults (e.g., fleas, female mosquitoes) obtain sufficient B vitamins from other dietary sources and are not dependent on symbiotic microorganisms. The chief evidence for microbial production of B vitamins comes from dietary studies, demonstrating that insects bearing the bacteria are independent of a dietary supply of B vitamins, while insects experimentally deprived of their bacteria require these nutrients. Supporting genomic evidence is available for the tsetse fly symbiosis. Specifically, the genome of *Wigglesworthia*, the mycetocyte symbiont of *Glossina brevipalpis*, has genes coding for the synthesis of pantothenate (vitamin B<sub>5</sub>), biotin (vitamin B<sub>7</sub>), thiamin (vitamin B<sub>1</sub>), riboflavin and FAD (vitamin B<sub>2</sub>), pyridoxine (vitamin B<sub>6</sub>), nicotinamide (vitamin B<sub>3</sub>) and folate (vitamin B<sub>9</sub>). B vitamin provisioning has also been proposed for various wood-feeding insects associated with mycetocyte symbionts.

### **Microorganisms and the sterol nutrition of insects**

Some insects obtain sterols from fungal symbionts. Bacteria generally have no capacity for sterol synthesis, and symbiotic bacteria do not contribute directly to the sterol requirements of insects. The principal evidence for the role of fungal symbionts in insect sterol nutrition comes from the composition of insect sterols: sterols of fungi are generally dominated by D<sub>5</sub>, 7-sterols such as ergosterol, and the presence of these sterols in the insect sterol profile is a firm indication of the fungal source. The leaf-cutting ant, *Acromyrmex octospinosus*, and the ambrosia beetle, *Xyleborus ferrugineus*, possess these distinctive fungal sterols, suggesting that they derive their sterol requirements from the fungi that they cultivate. Anobiid beetles *Lasioderma serricorne* and *Stegobium paniceum* also derive ergosterol and related sterols from their fungal partners (ascomycetes assigned to the genus

Symbiotaphrina), which are located in gut ceca, and they metabolize the fungal sterols to the dominant insect sterols, 7-dehydrocholesterol and cholesterol. The clavicipitacean fungi associated with the fat body of the planthopper. *Nilaparvata lugens* have a truncated sterol biosynthetic pathway due to nonsense mutations in genes mediating the terminal steps of the ergosterol biosynthetic pathway, resulting in the accumulation of ergosta-5,7,24 (28)-trienol, which is metabolized by the insect to 24-methylenecholesterol, cholesterol and other steroids.