

Chapter 2

Insect “Symbiology” Is Coming of Age, Bridging Between Bench and Field

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Abstract Insects are major contributors to natural as well as to man-managed ecosystems and largely bear on human affairs, both positively and negatively. They have varied microbial communities that provide them with nutritional benefits, enhanced defenses, and improved stress resistance. This review centers on nutritional contributions of gut symbionts to their insect hosts. It explores the complex relationships between symbiont, host nutrition, and host life cycle, summarizing some of the important developments in symbiotic science. It particularly focuses on Tephritidae fruit flies that cause enormous agricultural damage. It exposes the present knowledge on the symbionts of the tephritids *Ceratitis capitata* (the Mediterranean fruit fly) and *Bactrocera oleae* (the Olive fly) and recapitulates recent advances in the improvement of the performance of mass-reared sterile males used in sterile insect technique to combat the medfly.

2.1 Introduction

The founding myth of the San people in the Kalahari Desert is that the first human grew from a seed planted in a mantis by a bee. The Minoans believed in the Bee goddess, and in the Homeric myth, the Thriaie speak the truth after eating honey. Ancient Egyptians made dung beetles sacred and used bee wax as seals and in ship building; wax has also been lighting homes for centuries, and the silk fiber tied East to West through interminable roads for more than 3,000 years; butterflies and caterpillars were shown as symbols of transience and impermanence of earthly things: All these tell of the close association between insects and man. Insect lovers cannot but marvel at the contrast between the general apprehension against these

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arthropods we see today, and the reverence in which our ancestors seemed to hold them, leaving us mostly a positive, or at least revering view of these wonderful creatures.

Insects bear on human affairs. It was recently calculated that the economic value of pollination worldwide amounts to €153 billion (at a year 2005 value), or 9.5% of the total value of the world agricultural production used for human food (Gallai et al. 2009). Insects also provide enormous biocontrol, decomposition that increases soil fertility, and seed dispersion services estimated at tens of billions of dollars annually. Negative aspects of insects' impact on humans include diseases and agricultural pests. Insects and other pests destroy an estimated 40% of all crops worldwide, despite the yearly application of about three million tons of pesticides at a cost of more than \$25 billions (Pimentel 2009). Insects are also vectors of human, other animals', and plant diseases, including, naming a few, *Plasmodium* spp. (malaria), transmitted by mosquitoes, *Trypanosoma* spp. (tsetse), transmitted by *Glossina* flies, the tobacco mosaic virus and the tomato yellow leaf curl virus, both transmitted by the white fly *Bemisia* spp. Thus, in human perspective, insect activities range from positive to negative. Most of the pollinating activities provided by insects are essential, and may be defined as irreplaceable, or obligate, both for agricultural production and for the sustenance of ecosystems. Others may be facultative, such as natural biocontrol in agricultural fields, that can be replaced by chemicals – of course with economic, environmental, and health costs.

Insects may interact with the microbes, the microbiota they carry, in similar ways: they may enter cooperative interactions or suffer from detrimental ones. These interactions may be obligate or facultative.

Cooperative, mutualistic interactions based on nutritional contributions will be the topic of this review. I will review various insect-symbiont systems and parameters that may affect the type of symbiosis and will emphasize the role of the microbiota in pest fruit flies, insects that negatively affect humans. The chapter will end with a view on how microbes may be manipulated to reduce the damage toll of such nefarious insects.

2.2 P and S Symbionts

Associations between an insect host and its symbionts may be obligate or facultative. The greater the benefits accrued, i.e., the more host fitness parameters (including development, longevity, and reproductive fitness) are dependent upon the symbiont, the more stringent the association. In such cases, symbionts are mostly called primary (P) symbionts. Secondary symbionts (S) are mostly dispensable or even deleterious (Hypsa and Novakova 2009). While P symbionts may share long evolutionary histories with their hosts and are usually transmitted from parent to progeny, S symbionts may be more recent and are transmitted in a more “relaxed” manner, i.e., largely horizontally (Moya et al. 2008). Yet, heritable associations tend to become mutualistic, and the host is severely harmed by the elimination of

the symbiont (Baumann et al. 2006). Moreover, S symbionts can evolve to become obligate partners and establish a microbial consortium with the P symbiont or replace it (Wu et al. 2006; Toju et al. 2010).

Symbionts that are strictly transmitted vertically are obligate symbionts but not all obligate symbionts are solely transmitted vertically (Bright and Bulgheresi 2010). Strict symbionts are mostly intracellular endosymbionts, and are lodged within bacteriocytes (also called mycetomes).

2.3 Dietary Effects in Symbiotic Interactions

Symbionts can affect their hosts in many ways. Here, I will restrict to some aspects of dietary effects. Bacterial symbionts can improve diet quality when engaging in a mutualistic relationship and provision the host for nutrients lacking in its diet. Yet, the nature of the diet and the life cycle of the host seem to affect the type of symbiosis. Estes et al. (2009) proposed to interpret symbiotic relationships in insects along three variables defining a gradient of variability of the internal environment: (1) The host diet (monophagous versus polyphagous); (2) the host life cycle (hemimetabolous versus holometabolous); and (3) the location of the endosymbiont (intracellular versus extracellular).

Intracellular symbionts in holometabolous insects may face more challenges than intracellular symbionts of hemimetabolous insects: While both have to reach the progeny and pass through ecdysis, the former encounter the two, often ecologically largely different life stages of their hosts. To be conserved, the symbiont should be relevant to both stages: mutualistic in both, or mutualistic at one stage and parasitic at the other; or hitchhiking on particularities of the host's life cycle.

We will explore symbiosis along these lines, examining a few cases. This review does not intend to cover all aspects of dietary symbiotic interactions that certainly extend beyond straightforward nutrient provisioning but to provide a few lines of thought on some of their ecological and evolutionary aspects.

2.4 Nutrition-Based Symbiosis in Hemimetabolous Insects

Aphids and sharpshooters, sap-feeding insects, are at the one end of the spectrum: they feed on plant sap, a monotonous, nutrient deficient diet; they are hemimetabolous, with the nymphs and adults sharing the same habitat; and many aphids are monophagous, their endosymbionts are intracellular and as such are typically transmitted by transovarial mechanisms (Moran et al. 2008). Aphids, but also psyllids, whiteflies, mealybugs, and stinkbugs feed on phloem sap, a diet rich in sugar but poor in essential amino acids (Sandström and Pettersson 1994). Xylem sap provides an even poorer diet in sugar and amino acids and vitamins to

sharpshooters. Data from metabolic, ecological, genetic, and genomic experiments performed on these insects support the assumption that P symbionts provide essential missing nutrients to their hosts: For example, the P symbiont *Buchnera* spp. provides essential amino acids to their aphid symbionts (Douglas 2006); a remarkable duo of complementary capabilities is found in sharpshooters where the γ -proteobacterium *Baumannia cicadellinicola* supplies vitamins and cofactors and the Bacteroidetes *Sulcia muelleri* synthesizes essential amino acids (Wu et al. 2006). Both symbionts can be classified as P symbionts.

In sap-feeding stinkbugs, the symbionts are obligate (Hosokawa et al. 2006) and may also provide their hosts with nutritional supplements (Fukatsu and Hosokawa 2009). However, in contrast to aphids, these insects are mostly polyphagous, feeding on more than one plant species, thus enjoying a wider variety of resources (Kuechler et al. 2011), hold their bacterial symbionts in the lumen of midgut crypts, and transfer them to progeny by postnatal transmission mechanisms. Other polyphagous stinkbugs include the broad-headed *Riptortus clavatus* (Heteroptera: Alydidae) that feed on seeds and seedpods. Their *Burkholderia* symbionts fill midgut crypts, are not vertically transmitted but are environmentally acquired by the nymph at each generation (Kikuchi et al. 2007). Similar findings were obtained from 39 phytophagous species in the Lygaeoidea and Coreoidea stinkbugs (Kikuchi et al. 2011). The symbionts may provide vitamins and essential amino acids and fix nitrogen.

In sharp contrast to the sap-sucking stinkbug, the stinkbug *Cimex lectularius* (the bedbug, Cimicidae) solely feeds on blood. Blood is a resource low in vitamins of the B complex, as well as in several cofactors, and a *Wolbachia* symbiont provisions for the missing nutrients. Its elimination leads to retarded growth and sterility. *Wolbachia* usually affect their host reproduction, and in some cases were found to conditionally improve host fitness (Weeks et al. 2007; Brownlie et al. 2009). Yet, in the bedbug, *Wolbachia* seems to have shifted roles: it is found in specific mycetomes; is vertically transmitted by a transovarial mechanism; and has become an obligate mutualist possibly involved in vitamin biosynthesis (Hosokawa et al. 2010). Since over 60% of insect species may carry *Wolbachia*, the bedbug case may not be an exception (Hosokawa et al. 2010). This suggests that specific symbionts may not be as functionally compartmentalized as previously thought and may evolve to fill rather different niches in different hosts.

Termites are the subjects of intensive research as they offer fascinating insights into the role, effects, and evolution of symbiosis. All termites feed on nitrogen deficient diets, living off the decomposition of lignocellulose. Lower termites feed only on wood; higher termites obtain their food also from lignocellulose, either directly or indirectly (see below). The hindgut of lower termites supports large populations of anaerobic flagellates most of which harbor numerous epibiotic and intracellular prokaryotic endosymbionts: In *Coptotermes formosanus*, about 70% of the total rRNA of the gut community is found within the gut protists (Ohkuma 2008) with free-living prokaryotes (including those attached to the gut wall) making up a few percent of the total population (Ohkuma 2003). For example, in the cellulolytic protist *Pseudotrichonympha grassii* of *C. formosanus*, an

intracellular Bacteroidales (“*Candidatus* Azobacteroides pseudotrichonymphae”) endosymbiont accounts for the majority of the bacterial cells (Hongoh et al. 2008a). In *Reticulitermes speratus*, Elusimicrobiota (formerly TG1) bacteria hosted within the cellulolytic flagellate *Trichonympha agilis* represent about 10% of the gut prokaryotic population (Hongoh et al. 2008b). The former bacterium can fix nitrogen, and both synthesize amino acids and co-factors while utilizing cellulose degradation products as a carbon source and producing acetate, the energy and carbon source of the termite (Hongoh et al. 2008a, b).

Higher termites lack protozoan symbionts but still retain an enlarged hindgut, which host diverse prokaryotes. In wood feeding higher termites such as *Nasutitermes*, these bacteria provide cellulose and hemicellulose degrading activities (Tokuda et al. 2005), acetogenesis (Breznak and Switzer 1986), and potentially nitrogen fixation (Warnecke et al. 2007). Yet, the higher termites in general display only low nitrogen fixation activity and vary their diet by extensive foraging (Ohkuma et al. 1999). Most higher termites are humivorous, feeding on soil, using plant tissue, fungal hyphae, and other microorganisms, but mainly humus, as food sources (Brune 2006). Humus constitutes the stable organic fraction in soil. It is composed of fulvic and humic acids, humins, lignin-like, and peptidic material and may include sugar amines, nucleic acids, phospholipids, vitamins, sulpholipids, and polysaccharides, providing carbon and energy to the termites (Ji and Brune 2001). Consequently, soil eating termites may not need to obtain additional nitrogen by nitrogen fixation as the C:N of the material they ingest is quite low (9–14) (the termite body’s C:N = 4.3–6.9) (Tayasu et al. 1997). In fact, in place of the primary fermentation of carbohydrates that feeds the other termites, amino acids extracted from the humus peptidic fraction may constitute a major nutritive resource (Ohkuma and Brune 2006; Ji et al. 2000). In the context of this discussion, it is to be noted that soil is very heterogeneous, significantly varying in composition over time and space and can thus vary in nutritive value.

The feeding behavior of some wood feeding lower (like *Zootermopsis*, *Incisitermes* (*Kaloterme*s), and *Reticulitermes*) and higher termites (Termitinae, Apotermitinae, and Nasutermitinae) is also noticeable: They use rotting (thus colonized by fungi) wood or highly decayed wood mixed with soil, (Bustamante and Martius 1998; Bignell and Egelton 2000), and thereby obtain a more diverse and richer diet. Lastly, Macrotermitine termites grow a fungal comb of *Termitomyces* (Agaricales, Tricholomataceae) fungi. The fungus acts as an exosymbiont, decomposing pre-digested plant material (e.g., wood, dry grass, and leaf litter) and the fungal nodules as well as the mature parts of the fungus comb are used as a food source for the termite (Brune 2006; Wood and Thomas 1989). Although both fungus and termite contribute glycosyl hydrolases, glucanases, and glucosidases, the gut bacteria also appear to participate in the digestion of the lignocellulose material (Liu et al. 2011).

We thus can summarize that in termites symbiotic interactions span from intracellular (lower, wood, and decaying wood eating termites that hold bacteria-filled protozoa) to extracellular (higher, decaying wood, and soil eating termites in which the bacteria are in the gut lumen) to exosymbiotic, with part of the

microbiota acting as an “extended holobiont” (i.e. higher, fungal-garden tending termites). These associations are obligate (Sands 1969; Eutick et al. 1978): for instance, protozoan and prokaryotic symbionts are transmitted externally by coprophagy or trophallaxis. Finally, and most importantly, symbionts not only provide missing or limiting nutrients but also take part in food digestion.

2.5 Nutrition-Based Symbiosis in Holometabolous Insects

Tsetse flies belong to the Diptera, a phylum comprising about 20% of the insect diversity with more than 150,000 described species (Yeates and Wiegmann 2005). Diptera are holometabolous, i.e., they exhibit a larval and an imago stage, and experience complete metamorphosis during pupation between the two. This makes it possible for larvae and adults to differentially respond to selective pressures, to develop specific adaptations and lifestyles, to be ecologically different. Tsetse flies belong to the genus *Glossina*, feed on the blood of vertebrate animals, and are the vectors of trypanosomes. Although ontogeny includes three larval stages, pupation and an adult life, the tsetse life cycle is peculiar: a single egg is fertilized and retained within the uterus, where the larva develops. The larva is fed with a blood-derived diet very rich in proteins and lipids that is elaborated in the milk gland. It is the only food the larva eats, until the third instar when it crawls into the soil, stops feeding, and pupates to develop an adult body (www.fao.org/docrep/009/p5178e/P5178E04.htm). Therefore, while formally holometabolous, adult and larva do not experience great differences in nutrition, directly and indirectly feeding on blood, respectively. As in the bedbug, a bacterial symbiont, here named *Wigglesworthia*, may provide the deficient vitamins required for the fly’s nutrition and fecundity (Akman et al. 2002). *Wigglesworthia* is an obligate P symbiont hosted in a bacteriome and therefore intracellular, yet it is transmitted extracellularly to the intrauterine larva via the mother’s milk (Cheng and Aksoy 1999; Pais et al. 2008). Tsetse has a second symbiont (and *Wolbachia* as well, not discussed here), *Sodalis glossinidius*, found in midgut cells, the hemolymph, and other tissues, excluding the ovaries (Baumann et al. 2006). *S. glossinidius* appears to be evolving from a free-living to a mutualistic life style affects its host’s longevity and (Toh et al. 2006; Pais et al. 2008).

The family Tephritidae are dipterans. It is constituted of about 5,000 species in about 500 recognized genera (Evenhuis et al. 2008). A minority of these insects, circa 70 species, are known agricultural pests, inflicting significant damage to agricultural production. The family Tephritidae is well known for multiple invasions that cause important economic problems in fruit or vegetable crops in tropical and subtropical areas worldwide (Duyck et al. 2004). During invasion, newcomers can partially exclude and/or displace established fly populations. Many other dipterans may cause minor damage or are potentially harmful (White and Elson-Harris 1992).

The most deleterious genera *Anastrepha*, *Ceratitis*, *Bactrocera*, *Dacus*, and *Rhagoletis* infest fruits and cause billions of dollars of direct (crop loss) damage annually but much more when control measures are included. In addition, in the sub-family Tephritinae, the larvae feed on Asteraceae flower heads and often induce formation of galls (Headrick and Goeden 1994). The diversity of the Tephritidae is reflected in their anatomical adaptations: Many of these flies have digestive tracks containing specialized cavities or organs within which bacterial symbionts are hosted (Stammer 1929; Mazzon et al. 2008). The largely dominant populations in the Tephritidae belong to the Enterobacteriaceae, yet they differ between fly species (Jurkevitch 2011). Certain symbionts are readily culturable while others still remain uncultured.

Many of the fruit flies are polyphagous, feeding on different fruits and other food sources. Some of the fruit flies, such as *Ceratitis capitata*, the Mediterranean fruit fly (the medfly) can use a large collection of fruits as hosts and therefore affect many different crops. These fruits vary in protein ($0.86 \pm 0.59\%$ dry weight, dw) and carbohydrate, mostly sugar, content ($13.7 \pm 13.7\%$, dw) (means obtained from data on 37 fruits at: www.thefruitpages.com/contents.shtml; www.nal.usda.gov/fnic/foodcomp/search/). Consequently, the fruit habitat that medfly larvae experience varies but is invariably low in protein and high in sugar. Other fruit flies, such as *Bactrocera oleae* (the olive fruit fly), are specific and oviposit only in a particular host, in this case the olive fruit. Mature olives (the habitat of the developing larva) also contain little protein (circa $\sim 3\%$ dw) but a lot of oil (10–50% dw), carbohydrates (20–25, with sugar 4–10%, dw), and sterols (3–5% dw) (Zamora et al. 2001; Maestro Duran 1990). In contrast, at the adult stage, both flies appear to feed on sugar rich diets such as fruit juices, honeydew, nectar, and fruit and plant exudates, as well as on microorganisms (Drew and Yuval 2000), and occasionally on bird droppings and pollen (Christenson and Foote 1960; Drew and Yuval 2000). Thus, the medfly and the olive fly’s diets are rather different at the larval stage and less so at the adult stage. As a result, it can be expected that if symbiotic associations occur during larval development and during adult life, they should be adapted to the different requirements of their hosts. According to the holobiont model (Zilber–Rosenberg and Rosenberg 2008), changes in adaptive traits may be associated with changes in the microbiota: A different dominant symbiont or a different community between the two stages – and between the medfly and olive larvae may thus be expected.

The microbiota associated with the medfly is essentially composed of Enterobacteriaceae and include *Citrobacter*, *Enterobacter*, *Klebsiella*, *Pantoea*, and *Pectobacterium* as dominant genera (Behar et al. 2005, 2008a; Ben Ami et al. 2010) and of *Erwinia dacicola*, *Enterobacter* sp. or *Acetobacter tropicalis* in the olive fly (Capuzzo et al. 2005; Estes et al. 2009; Kounatidis et al. 2009). Additionally, a cryptic but stable community of pseudomonads is found at very low concentrations in the medfly’s gut (Behar et al. 2008b).

In the medfly, most of the populations are nitrogen fixers, pectinolytic or both, providing atmospheric nitrogen to adults and larvae and degrading the pectin in the infested fruits, potentially increasing sugar availability to the growing larva (Behar et al. 2005, 2008a). Thus, in the fruit, the symbionts can act externally to

the insect's body. In addition, the gut microbiota may help recycle nitrogenous waste products into usable compounds such as uric acid and ammonia. Uricase, an enzyme that degrades uric acid into allantoin, may be produced by *Enterobacter* sp. present in the medfly's gut (Lauzon et al., 2000). Allantoin can be further processed to urea. In turn, Enterobacteriaceae like *K. oxytoca* or *Enterobacter gergoviae* can transform urea into ammonia (Zinder and Dworkin 2000).

The medfly's microbiota was shown to affect fitness parameters. Antibiotic treated males (thus bearing a much smaller gut microbiota) exhibited reduced mating competitiveness, as measured in a mating latency test; in females, the oviposition rate was significantly altered by antibiotic treatment (Ben Yosef et al. 2008a).

In the olive fly, the contribution of the gut bacteria in providing essential amino acids to the adult was demonstrated (Ben Yosef et al. 2010). It was postulated that bacteria associated with the larva actively detoxify the antimicrobial compounds produced at high concentrations by the green olive (Amiot et al. 1989; Ryan et al. 1999).

Although the same species are present at different stages of the medfly life, their abundance is significantly altered during ontogeny as reflected in the shift in community structure from parent to egg, larva, pupa, and adult (Behar et al. 2008a). The medfly symbionts appear to be extracellular at all stages and to be distributed in all sections of the gastrointestinal track. Gut colonization occurs stepwise, as bacterial foci expand in the gut during larval growth, and after pupation, during adulthood (unpublished data).

In the olive fly, the apparently same *E. dacicola* symbionts are intracellular at the larval stage, residing in the esophageal bulb, and extracellular, found in the intestinal lumen of the adult, transiting from the former to the latter as bacterial masses (Capuzzo et al. 2005; Estes et al. 2009). Thus, as in the medfly, the same community is found at both stages but the specific populations are found in different organs during the larval and adult stage and may differ in their relative abundances.

In both flies, egg smearing during passage in the ovipositor seems to be the mechanism by which the symbionts are transmitted to the next generation (Estes et al. 2009; Lauzon et al. 2009; Behar et al. 2008a; unpublished results). How can vertical transmission be guaranteed when nutritional needs and therefore the contribution of symbionts may change during the life cycle of the host?

Versatile, multivalent contributions by the same symbiont to both stages, as may occur in the olive fly, can offer a means to assure transmission to the next generation. "Ecological hitchhiking" may be another strategy: as seen above, the same species are found at all stages of the medfly's life cycle but differ in abundance. During oviposition, a mixed inoculum is introduced into the fruit, where it is differentially amplified. Larval, and then adult colonization provide subsequent bottlenecks that further and sequentially alter community structure (Behar et al. 2008a). The relative roles of competition, cooperation, local adaptation to the life stage, interactions with the immune system, or else, to shape the

community are not known. Yet, the diversity at the strain level, the variability between individuals and the large population size of the gut microbiota (Behar et al. 2005, 2008a, b) help assure that a diverse community may graduate to the next generation. Stochastic effects may lead to eggs bearing different inocula. This, in turn, can provide an advantage when the environment in which the larva develops may change from generation to generation, as in a highly polyphagous insect like the medfly. Holding such a diverse community at the adult stage may come with a cost: Antibiotic-treated, nutritionally stressed flies lived significantly shorter lives than control flies or flies fed a full diet (with or without antibiotics) (Ben Yosef et al. 2008b).

The gut of Lepidopteran larvae do not contain specialized structures such as diverticula, and the contribution of microorganisms to nutrition and digestion was thought to be negligible (Appel 1994). In a recent study, a large (3.10^{11} cfu.ml⁻¹) community of anaerobic bacteria was found in the gut of *Bombyx mori* larvae, the silkworm that feeds on mulberry leaves (Anand et al. 2010), suggesting that they may play a role at the larval stage. A few isolates were characterized and classified as Enterobacteriaceae, mainly *Klebsiella*, *Citrobacter*, and *Erwinia*. Most were able to degrade cellulose, xanthan, and pectin (Anand et al. 2010). Yet, the abundance of the carbohydrate degraders in relation to the total culturable community was relatively small, so their contribution to digestion of the carbohydrates is uncertain. It is interesting that the microbiology of such a historically, culturally, and economically important insect has not received more attention.

Predatory holometabolous insects may be at the other end of the symbiotic spectrum: they exhibit clearly separated two-stage life cycles and feed on a complete diet. Intracellular host reproduction manipulators are present in predatory (including holometabolous and hemimetabolous) insects (Dunn and Stabb 2005; Hoy and Jeyaprasaksh 2005; Dedeine et al. 2001). However, the existence of diet upgrading in such insects has been questioned (Gulian and Cranston 2010). Nevertheless, bacteria may contribute an extended version of a digestion service to their hosts. In Myrmeleon, the ferocious appearing larvae are called antlions, and live off the body fluid of their prey for 1–3 years. The prey is paralyzed by an injected homologue of the GroEL chaperone that acts as a toxin and is produced by *Enterobacter aerogenes* in the predator’s salivary glands (Yoshida et al. 2001). Interestingly, antlions have a discontinuous gut in which the midgut ends as a blind sac rather than being connected to the hindgut, and Enterobacteriaceae largely dominate this gastrointestinal track (Dunn and Stabb 2005). Enterobacteriaceae as well as *Bacillus* spp. were isolated from the crop and from the regurgating fluid and were shown to be insecticidal. Furthermore, bacterial cells may be injected into the prey by the predator (Egami et al. 2009; Nishiwaki et al. 2007) and proteases secreted by these bacteria may be involved in the liquification process of the prey content, i.e., they participate in extra-oral digestion (Egami et al. 2009). It remains to be seen what relation the relatively short-lived (20–45 days) adult antlions that feed on small flies, fruit, and honeydew (Burton and Burton 2002) entertain with their yet unknown bacteria.

2.6 Putting Symbionts to Work Against Insect Pests

In the medfly, the effects of the microbiota on longevity are intricate. As seen above, gut bacteria inferred a cost to nutritionally-stressed hosts (Ben Yosef et al. 2008b). However, the addition of a mixture of Enterobacteriaceae at high concentration to sugar-fed flies increased longevity. Substituting this inoculum with a low concentration of a *Pseudomonas aeruginosa* strain isolated from the fly gut lead to rapid death (Behar et al. 2008b) suggesting that the growth of these populations has to be tightly restricted. Symbionts have been shown to protect their insect hosts against pathogens (Brownlie and Johnson 2009) but they also have a role in keeping a proper balance between components of the gut microbiota. Commensal bacteria and the host immune system work in concert to modulate immune tolerance to commensal bacteria and respond to bacterial infection (Lhocine et al. 2008; Ryu et al. 2008). In the medfly, the dominant enterobacteria may help control the size of the pseudomonad community that however may not be essentially detrimental. In a sterile insect technique (SIT) facility mass-producing sterile males, a high level of pseudomonads were found in healthy flies (Ben Ami et al. 2010). SIT is an environmentally friendly approach that calls for the release of sterile males in the field to compete for mating with females against wild males, thereby reducing pest density (Hendrichs et al. 1995). SIT can lead to eradication under certain conditions (Vreysen et al. 2000). A main drawback of SIT has been the rather low mating performance of sterile males that compete poorly against wild males. Sterile males have to be frequently introduced in large numbers into the field, resulting in high operational costs.

In SIT, pupae are irradiated to render the male sterile, and this was shown to significantly alter the composition of the insect gut's microbiota (Ben Ami et al. 2010). In not irradiated males, *Klebsiella* spp. constituted about 20% of the total gut population in contrast to only 4% in irradiated males. Ben Ami et al. (2010) demonstrated that under laboratory conditions, the introduction of a *Klebsiella* strain isolated from a wild medfly into the irradiated male improved the fly's mating performance in a mating latency test, illustrating that gut bacteria can affect male attractiveness. How females gauge males and which cues are affected by the male microbiota is unknown. Sharon et al. (2010) showed that in *Drosophila*, flies performed assortative mating based on the type of gut bacteria they had, and the female flies also displayed different cuticular hydrocarbon sex pheromones. Yet, in the medfly, males are the ones that produce sex hormones suggesting that different mechanisms affecting mating exist in different flies.

In a recent paper, Gavriel et al. (2011) described scaling up of the probiotic approach developed by Ben Ami et al. (2010). Probiotic-treated sterile flies successfully competed against control, untreated flies or against field-caught wild flies for mating with females, in settings ranging from 100 l tents to ~8 m³ cages with an enclosed citrus tree mimicking field conditions. Mating with probiotic amended flies also significantly reduced the frequency of female remating with wild flies.

These studies offer a first proof that manipulation of a gut symbiont may be used to improve pest control.

The field of insect-symbiont interactions is yielding fascinating discoveries. At least some of these findings will certainly find their way to help improve the human lot.

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