Microbes and Insects: Effects, Prospects and Why We Should Respect

Introduction

I decided to write on insects because I've been interested in them ever since I was young. This includes spiders and similar non-insect creatures. I find them absolutely fascinating to watch and I even used to play around with small beetles and bugs I found around the house (I still do). Except for cockroaches, which somehow I am deathly terrified of and I'd pick getting eaten alive by spiders over touching a roach any day. With that said, I am deeply disheartened by the general public's disdain and lack of knowledge regarding insects. Understandably, the appearance of many insects is enough to put many people off. The fact that many have wings and can fly can evacuate rooms of seemingly calm individuals. Too many times have I encountered a beetle or strange-looking bug with someone and the first thing I hear from them is to kill it. I hope that one day I can change people's perception of bugs, and let them know that most insects can barely hurt us, and those that can, usually attack as a last resort. There is a beautiful diversity of insects out there, being the most speciose group of eukaryotes, and they are all around us; we just don't open our eyes wide enough, or even try to. Though as much as I'd like to, I will not really delve into that. I will instead be focusing on another group of the most speciose and misunderstood organisms out there – microbes, and their interactions with insects. Being so tiny that we cannot see them with our naked eye, it is no wonder that such a group is so poorly understood, with many people thinking "microbes = bad". Here, we will explore the effects of microbiomes in insects and what they have done to influence insect survivability and behavior. Next, we briefly look at some ways we can harvest microbes from insects for their uses, and finally, as with all organisms, there are favorable ones and others deemed as harmful. We will learn how certain microbes influence insect pests and what we can do about it. Through this read, I hope some (including me!) can understand better about the relationships between microbes and insects, what they can do for us, as well as to not underestimate their impacts on humans and the ecosystem, despite seemingly not even being seen at all! They are much more complicated than they look, and I hope readers will leave with a greater appreciation of bugs, not to mention microbes as well.

Microbiomes of Insects

There are many kinds of microbes associated with insects, from bacteria and viruses to archaea and fungi (Gurung, Wertheim & Salles, 2019). They usually inhabit the insect exoskeleton (outer surface) and the gut, but some are able to breach into the hemocoel (the insect equivalent of our circulatory system transporting blood), to inhabit cells as well. Microbiomes of insects generally function to improve nutrition, confer protection, detoxify, and alter behavior (Douglas, 2015). Of course, they don't do this for free, as living with the insects tends to improve their own fitness and quality of life as well. Microbes can obtain free nutrients and a home with optimum conditions of temperature and humidity, away from the harsh world outside. In this way, both organisms hopefully can live long enough to reproduce with healthy young. This is known as symbiosis, with both partners helping each other out to survive, though some microbes can be pathogenic as well, harming the insect for its own benefit. If only humans could "symbiose" just as well with each other, right?

Microbiomes of Insects – Nutrition

Starting with nutrition, microbes in insects can serve to improve nutrition uptake in them. A good example would be of the bacterium *Buchnera aphidicola* in pea aphids (Sanchez-Contreras & Vlisidou, 2008). Aphids are tiny insects living in colonies that use piercing mouthparts to suck plant sap. Pea aphids look like bulbous green teardrops with legs, and they definitely do not taste like peas. The bacteria lives inside cells of the aphids, of which the bacteria is now known as a primary endosymbiont. The aphids, like many insects, have specialized diets and only feed on plant sap, which is abundant in sugars but low in essential amino acids for protein building and growth. This is where the bacteria comes in, synthesizing such amino acids for its host whilst utilizing some sugars for itself; a merry relationship! Amazingly, this partnership seems to have been established up to 70 million years ago, and both species have even followed in each other's footsteps to diverge from other species together, a process called cospeciation. They are also obligate, which means they cannot live without each other. Furthermore, secondary endosymbionts exist as well, such as the bacterium *Hamiltonella defensa*. It is not obligate like *Buchnera aphidicola*, and lives in the aphid hemocoel ("blood") instead of aphid cells. It synthesizes the amino acid tryptophan for its host, and promotes the healthy development of the aphid to reproductive maturity.

Other examples include that of the tsetse fly, containing bacterial symbionts which help supply vitamins and important nutrients to their host (Sanchez-Contreras & Vlisidou, 2008). The fly cannot synthesise such food on its own as it only feeds on blood (unfortunately for us), which happens to be high in protein but low in sugars this time, in contrast to the aphids. This occurs similarly in some flies, lice, beetles and hemipterans (insects with sucking mouthparts) as well (Douglas, 2015).

Not only can microbes exchange nutrients with their host, they can also directly assist digestion of food taken up by the host. Bark beetles eat wood, however their digestive systems cannot naturally process wood very well. The presence of a fungus in their gut, specific to each species, helps to first break down the wood into nutrient-rich material (Steinhaus, 1940). They even culture these fungi in their beetle burrows! Some wood-eating termites also similarly possess protozoans in their gut to digest wood.

Microbiomes of Insects – Protection and Detoxification

Other than assisting nutrition, microbes increase the fitness of their hosts as well, through means of protection from attackers such as parasitoids, or from environmental conditions.

A very interesting example is of the pea aphids again, this time with the friendly parasitoid wasps (Gurung, Wertheim & Salles, 2019). Parasitoid wasps look quite domineering, especially when you see their stingers, which can grow up to several centimetres long, sometimes even longer than their own bodies! Luckily for us, those are actually not stingers, but lengthened ovipositors, or egg-laying structures, and they do not use it to sting in defense. If you were an aphid however (or look like one), then don't count yourself so lucky, because these wasps will actively seek you out to inject their eggs inside your body. You will then be slowly consumed from the inside by the hatching larvae, and your body used and abused without consent. The reproductive organs of these wasps contain certain viruses, that when injected into the aphids, serve to suppress their immune systems to further make sure they cannot fight back. However, not all is lost, as some pea aphids with the previously mentioned secondary endosymbiont *Hamiltonella defensa* contain phages that protect them from parasitoid viruses of these wasps. This results in an arms race, where aphid and wasp subconsciously fight over many generations to slowly adapt over each other's offenses and defenses; a race I would personally like to be in the audience of, if I could only live a million years more.

Other such examples of protection include the production of polyketide toxins by *Pseudomonas* bacteria in certain rove beetles (Douglas, 2015). This toxin flows in their hemolymph, and causes burns or skin irritations to predators that attempt to munch on them. Do think twice before you try and squash a beetle next time!

Furthermore, microbes help detoxify insects, especially those on plant diets (Douglas, 2015). Certain groups of beetles and ants harbor yeast and bacterial gut symbionts that degrade plant toxins when digested. Similar to wasps and aphids, plants and herbivorous bugs are involved in their own war, with plants trying to avoid being eaten and the bugs overcoming plant chemical defenses. Likewise, resistance to pesticides may also exist in insects that contain bacterial strains that have evolved resistance over time.

Microbiomes of Insects – Behavior and Evolution

We will explore the influence of microbes on insect behavior in terms of sociality. Have you ever wondered how insects became social? How did living and doing things by yourself, slowly evolve into doing things as a group? Microbes are a possible influence of social behavior in insects, though it should be noted that there are other factors at play, and that correlation may not equate causation (Onchuru et al., 2018). It is already known that microbes provide immeasurable benefit to their hosts, but we haven't delved into how these helpful symbionts are passed on to each other or to offspring. In solitary insects, they sometimes resort to depositing faeces and secretions containing beneficial microbes near their eggs or young (my parents did this to me too). When the eggs hatch, they may obtain the microbes, however there is an obvious chance of this transmission failing. There exists more direct ways of transmitting microbes to others or to offspring. One such method is coprophagy, the eating of each other's poop. After all, good things must be shared, right? Other ways include trophallaxis, the mouth-to-mouth exchange of oral fluids, of which many species of bees do to transfer gut microbes to their young. Of course, when I try to justify the benefits of trophallaxis to girls, they always end up fleeing, but their loss I guess. Jokes aside, simply sharing the same environment such as shared host plants of herbivorous bugs is also enough to encourage high sharing of microbes. Thus, it is a possibility that insects who accidentally engaged in such direct behavior managed to obtain more beneficial microbes than their solitary mates, and were able to survive to fertility and reproduce with young that continued such behavior, slowly evolving sociality! It should be noted though, that sociality comes at a cost, of which harmful parasites and pathogens are likely as well to be transferred within insect communities. For sociality to evolve, the benefits have to outweigh the costs. An example that furthers the case of microbes influencing sociality is that of a phenolic compound produced by the bacteria *Pantoea agglomerans* in the guts of desert locusts (Douglas, 2015). This causes them to behave gregariously and group up, thus increasing chances of mating whilst providing safety in numbers, which allows both the bacteria and locust to expand their populations. It is marvelous to see how evolution works!

In extreme cases of sociality, such as eusocial bees living in a group, there are often acts of altruistic behavior. In bees, work is efficiently divided without complaint. There will be worker bees to collect pollen, a queen bee solely for reproduction and many more castes and roles. If a predator were to attack the hive, worker bees would rush to sting the predator, and many would die thereafter from being disemboweled. So why would these heroes of the nest sacrifice themselves for the greater good of the colony like that? They don't seem to receive any direct benefit whatsoever, especially since the primary goal of many insects is to propagate their own genes. In a model proposed by Lewin-Epstein et al. (2017), they found that microbes can indeed influence altruistic over self-beneficial behavior. Since the hive survives while the heroes die, the heroes' sisters can still propagate their genes to give rise to new young, keeping the blood lineage going. If altruistic behavior did not occur, the hive would have potentially lost, and the bloodline would surely end. We humans could definitely learn a thing or two from these eusocial organisms.

Microbiomes of Insects – Pathogens and Complex Interactions

With the good of microbes, there always exists the bad, and this is no different for the insects. Harmful pathogens exist everywhere that insects can pick up. One such example is the bacterium *Serratia marcescens*, extremely common in many invertebrates (Sanchez-Contreras & Vlisidou, 2008). *Drosophilia* flies infected with this can die within a day, where the bacteria crosses to and infects the gut whilst dodging immune response. *Pseudomonas entomophila*, when ingested by insects, induces an immune response that results in blockage of food uptake and a resultant death by starvation. In extreme cases, parasitic microbes can wipe out entire colonies of insects, especially eusocial ones. It is nice, yet morbid to know that it is not only humans that can experience such "plagues" similar to the Black Death.

With this, it is important to note that inside an insect, there exists many complex interactions between microbes, as well as with their predators and prey. Such was demonstrated previously by the aphids, plants and parasitoid wasps. Aphid is trying to eat plant, wasp is trying to lay eggs inside aphid, and everyone is trying to evolve their own microbial offenses and defenses as well. In three-way interactions between microbes, plants and insects, microbes can serve to deter insects away from plants, but attract them as well (Noman et al., 2020). By understanding these multi-layered interactions better, we can then most efficiently improve our understanding and employ applications of ecology to ourselves.

Applications of Microbes in Insects

Insects are wonderfully diverse, and their equally diverse microbiomes present a possible solution to issues such as the alarming rate of antimicrobial resistance in the world. Many natural sources of antimicrobials have been neglected in favor of artificial chemistry in order to fight this resistance, but the numbers of novel discoveries have been dwindling. By studying microbial-insect interactions, many compounds can be elucidated, providing much insight on metabolic pathways that can be replicated to produce useful bioactives (Beemelmanns et al., 2016). Some examples include defensive symbionts from wasps, fungus-growing beetles and ants, and insect-infecting fungi. The antimicrobial *Streptomyces* bacteria has much undiscovered potential in antibiotics and antifungals, especially when sourced from insects, more so than from plants and soil (Chevrette et al., 2019). If we can combine our efforts from natural and artificial methods, perhaps then can we successfully tackle the issues of disease and microbial resistance in our world, not to mention helping conservation efforts.

Furthermore, gut microbiomes from insects have certain implications for biotechnology. By studying microbial genomes from insect digestive systems, we can discover many novel genes and enzymes that serve purposes such as digestion and microbial communication

(Krishnan et al., 2014). Microbes from termites have been identified and harvested to convert wood into energy-conserving biofuels, while vitamins can be produced from the identification of bacterial genes within a tsetse fly. With the massive diversity of bugs out there, there is a whole world just waiting to be discovered by us!

Microbes and Insects as Pests

Unfortunately, the issue of insects as pests in the world is a rather large one. Invasive insects impact the economy and environment of places where they are introduced to. An interesting example is of the silverleaf whitefly. These flies with white wings and yellow bodies surprisingly only turn invasive outside of their native range depending on what they consume or are infected by (Lu, Hulcr & Sun, 2016). Consuming plants infected with Tobacco curly shoot viruses renders them more long-lived and extra fertile, allowing them to invade more regions of Southern China to destroy crops. Meanwhile, being infected with *Rickettsia* bacteria makes them similarly faster developing and invasive in the Southern United States. In other cases such as the Sirex woodwasp and its fungal mutualist *Amylostereum areolatum*, they function together to destroy valuable pine trees. When depositing eggs into pine trees, the associated fungus degrade the pines along with the wasp toxins, providing a suitable environment for the wasp larvae to grow. Sometimes, complex interactions between multiple microbes and insects overall serve to increase invasiveness in an area. Thus, it is important to study in depth these complex interactions between plants, bugs and microbes in order to efficiently tackle problems regarding agricultural pests. Imagine working your way up to grow fields of crops for a big payout, only to have them destroyed by these damned creatures that you can barely even see.

Next, insects carrying microbes also serve as vectors of disease. This is a huge problem for people all around the world, from kissing bugs transmitting protozoans causing Chagas disease, to sleeping sickness caused by similar symbiotic protozoans of tsetse flies. Despite this, who else do we turn to to solve these problems for us, but other microbes! There are many ways to do this, and here I will give one example of how we do in a local context. Dengue is a huge problem is Singapore, and the virus is carried by mosquito vectors, such as *Aedes aegypti*. However, when these mosquito eggs are injected with Wolbachia bacteria, resultant males that are reared will grow up to mate unsuccessfully with uninfected female mosquitoes (Sanchez-Contreras & Vlisidou, 2008). Singapore's Project Wolbachia (NEA, 2020) have implemented this in certain areas rather successfully (I can testify; I live in one of these areas and am personally dengue-free). And not to worry, the release of Wolbachia-infected males is not a cause of concern. They do not bite, and the Wolbachia bacterium is perfectly safe for humans! In other countries, ongoing projects regarding yeast fermentation odors and chemical signaling via pathogens also serve to eliminate pests and vectors (Becher, 2020)

Conclusion/Reflection

In conclusion, the microbiome of insects consists of many mutualistic interactions that benefit their host, such as improving nutrition, conferring protection and potentially influencing behavior for evolution to occur in the long run. Pathogenic microbes exist to harm as well. Microbes in insects can be studied for harvesting of useful bioactive compounds, with applications in biotechnology too. The issues of invasive bugs and bugs as a vector of microbial disease can be tackled in many clever ways. However, in order for us to fully harness the usefulness of insect microbiomes for their benefits, we must make sure to see the wider picture, including interactions between plants and predator-prey, as well as with any extra associated microbes.

I hope that through this read, you will have learnt some interesting things about microbes and bugs, and can perhaps start appreciating them a little bit more! It is wonderful how nature managed to evolve such complex relationships in these tiny organisms that most wouldn't bat an eye about when going about their daily routines. I started writing this with some initial understanding of insects, but honestly zero knowledge of their microbes and associations. Some really interesting things that I learnt was of the "arms race" between predator and prey. An individual may not really just be in a single race, rather they can be involved in multiple at the same time! Trying to overcome your plant preys' defenses, while trying not to be overwhelmed by predators' offenses must be a lot of work, in the long run of evolution. Learning that microbes, of all things, could possibly have contributed to social behavior and sacrificing "for the greater good" was really cool. It made me wonder, if somehow microbes have influenced human evolution in such ways as well, and possibly other social or even cultural behaviors? Perhaps we are not so different after all. Realising that insects suffer from just as much disease and pathogens as us also humanizes them a little. I cannot imagine how many millions of insects which must be dying everyday, not to mention entire colonies being wiped out. But then again, that is the fragility of life, and we are of no exception. So it would be good to treasure what we have now. Anyway, I did notice that most insects I encountered during my research were that of certain major groups, such as beetles, hemipterans (aphids etc.), ants and wasps. It made me think, if the impacts of other lesser-known bugs were perhaps just not as large as these groups, and if that undermines their importance. Though this could very well be attributed to the major groups of insects being some of the most diverse and abundant bugs found, it may be no wonder that the amount of research on them is proportionally higher as well. I aspire to find out one day, or even lead the research on these lesser-known and underappreciated bugs. Through this, I appreciate microbes and insects much more now, and I understand to not underestimate the potential benefits, and harm that they can bring to human societies. I will continue to work on and further my knowledge of bugs throughout the rest of university, and hopefully educate people, one at a time, on these amazing societies happening all beneath our feet.

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