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Review Paper

The Benefits faced by Aphids due to their Endosymbionts

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Abstract

Aphids enter a symbiotic relationship with bacteria which holds influence on their ecological characteristics. Although widespread in nature, investigation on their symbiotic relationship is yet to go on full-fledged. Both the obligate and facultative endosymbionts affect the life history traits of the aphids. I discuss the beneficial effects that the endosymbionts provide to their hosts and point out the questions based on which research can proceed further with respect to aphid-bacteria interactions. I finally conclude with some questions on this aspect and by pointing out the avenues which we can work on to develop a deepened insight of the evolutionary ecological traits.

Keywords: Aphids, Endosymbionts, Evolutionary Ecology, Benefits, Costs.

Introduction

Insect- microbe interaction is a key driver of the ongoing processes of nature. Such interactions are in the form of mutualism, commensalism and parasitism. The one between the aphids and their internal microbes which are located in the bacteriocytes inside the hosts is symbiosis by which the aphids act intimately with their bacterial partners. The endosymbionts have led their hosts to develop ecologically important traits. And the study of such symbiotic relationship has grasped the attention of the researchers. Aphids harbour multiple bacterial symbionts which are classified as primary and secondary endosymbionts. These symbionts appear to impose costs on their hosts for the maintenance of their population as well as provide benefits to their hosts pertaining to nutrition, defense, stress control and reproduction[1-3.]

Most of the studies on aphids have been in the context of invasion, damage, dispersal strategies, suitable climate, spatial distribution, diversity, vectors, reproductive strategies, parasitoid attacks. However, past few decades have seen the research of the aphid- bacteria symbiosis with respect to the evolutionary ecological traits. Both the primary and the secondary endosymbionts are vertically transmitted along with the genes of their hosts. Additionally, horizontal transmission between the same or different species of the symbionts occur that might change the ecological evolutionary dynamics- a similarity with the bacteria mediating antibiotic resistance[4].

The wheat aphid *Diuraphis noxia* is second to the pea aphid *Acyrthosiphon pisum* whose genome has been sequenced till date[5]. It is the first of a phytotoxic insect too, to be sequenced, the knowledge of which would help in unraveling the evolutionary dynamics of its influence on the host plants and phytotoxicity. Further, the sequenced genome of primary symbiont *Buchnera aphidicola* reveals its size to be decaying every generation owing to the bottlenecks[6]. Factors that shape the bacterial communities tend to be rate of horizontal transmission, interaction between the genotypes of the hosts and the symbionts and with the environment as well. A number of studies addressing the issue indicate that the aphids harbouring the bacterial symbionts are both positively and negatively affected.

In this review I give an account of the various benefits that the bacterial symbionts have conferred on their aphid hosts. Work on aphid- bacteria symbiosis is yet to take its pace and more data related to their ecological traits is required for a deeper insight.

Nutrition

Buchnera aphidicola remains enclosed in specialized cells bacteriocytes inside the aphids and furnishes the host with the amino acids. The genes trpEG and the cluster leu ABCD of the bacteria, which are present in different plasmids, are responsible for conferring the nutritional benefits of tryptophan and leucine[7]. Furthermore, a number of amino acid transporters are present on the surface of the bacteriocyte membrane that regulate the exchange of the amino acids between host and symbiont[8,9]. The presence of metabolic enzymes such as ATP synthase and glyceraldehyde- 3-phosphate dehydrogenase in *B. aphidicola* suggests the presence of ATP synthesis and glycolytic pathways which possibly is tightly linked to the energetics of the host[10]. But we are yet to know if these are the properly conserved domains across strains and species and whether these proteins face any kind of change in their domains with the variation in geography as well as climate.

Heat

Not only the host, the endosymbionts too benefit from each other and this is proved by a study conducted by Burke G et al in 2010 where on subjecting the pea aphid *Acyrthosiphon pisum* to heat stress (temperature in the range 20°C-39°C), the associated facultative bacteria *Serratia symbiotica* provided a shielding effect towards the primary bacteria *Buchnera* as well as contributed towards overall fitness of the aphids[11]. It was hypothesized that *S. symbiotica* after their lysis aided in metabolite delivery in such stressful conditions and this gives rise to a possibility of the chemical activation of the necessary compounds involved in shielding following heat mediated lysis. Hence the symbionts directly or indirectly provide protection to their hosts. Genome sequencing from the recently evolved aphid symbiont *S. symbiotica* exhibits massive genomic decay; a fact that all vertically transmitted bacterial symbionts undergo a reduction in genome size over time. So the genes that influence this thermal tolerance possibly must not be undergoing the decay. Or even if they are likely being deleted then any kind of costs associated with the hosts may be the reason. It was reported that a single base mutation in the ibpA gene that encodes for the heat shock proteins of *Buchnera* in the aphids *Acyrthosiphon pisum* and *Schizaphis graminum* affects the aphid following the heat response[2]. We may look for this gene in other species too and test the influence of other genes on ibpA- an aspect which will be quite difficult to investigate owing to the possible gene rearrangement and decay they undergo. Aphid hosts when subjected to heat stress for a short duration as juvenile had low fecundity in presence of the mutant ibpA in *Buchnera*. However, under cool conditions the bacteria confer benefits on host fitness. Not only the point mutation in the bacterial gene but temperature too plays pivotal role in the host ecology. Besides, future studies regarding heat stress should also include the fact of examining the different stages of the aphids.

Parasitoid attack

In order to test the resistance conferring role of the symbionts against parasitoid attacks the aphids were usually cured of the target endosymbionts on one hand while injected with the same into the non-hosts or other host members on the other hand. Oliver et al in 2003 had investigated the role of facultative symbionts in conferring protection against parasitoid *Aphidius ervi* (North America)[12]. Following artificial injection of the uninfected aphids with the secondary bacteria, although, they were still susceptible to parasitoid attack they did not support their persistence. A strain of *Regiella insecticola* isolated from Australian clones of *Myzus persicae* was reported to protect the host against the parasitoids[13]. The *R. insecticola* strain derived from the affected aphid was transfected into another strain of the same species as well as *Aphis fabae* and exposed to the parasitoid *Aphidius colemani* for a fixed time interval. They concluded that the cured aphids became susceptible whereas the transfected ones became resistant. The facultative symbiont *Hamiltonella defensa* potentially provides resistance against *Aphidius ervi*. Oliver et al in 2009 tested five of the isolates that showed varying level of resistance from 19% to 100%. Additionally, A. pisum genotype did not play any role in mediating resistance in this experiment. Further, the two endosymbionts *H. defensa* and *S. symbiotica* in *A. pisum* exposed to *A.* ervi conferred a greater resistance together in comparison to those harbouring either of them[14] . Toxin encoding lambda bacteriophage *A. pisum* secondary endosymbiont (APSE) is responsible for development of protective phenotype in *H. defensa* against *A. ervi*[15, 16]. Despite this, such events of superinfection are rare as observed in the survey of *A. pisum* secondary symbionts in northern Utah possibly owing to fitness costs, longer generation time, lower weights at adulthood. *H. defensa* confers a general resistance against the parasitoids *Aphelinus abdominalis* and *Aphidius rhopalosiphi*. But *R. insecticola* failed to give resistance to *Sitobion avanae* (F) against *A. rhopalosiphi*[17]. Thus a question arises here on the capability and the extent of the different strains of the parasitoids to attack their respective prey.

Fungal pathogen

According to Scarborough et al *Regiella insecticola* prevents sporulation of the pathogenic fungi *Pandora neoaphidis* on the cadavers of *A. pisum*[18]. This was further substantiated by Godfray et al in 2013 who found out that *Rickettsiella* or *Rickettsia* provided immunity to *A. pisum*[19]. Another strain of *R. insecticola* is reported to defend the host against *Zoophthora occidentalis*[20]. *Spiroplasma* and another isolate of *Regiella* could not prevent the fungal invasion but were capable of delaying the death and sporulation. *Spiroplasma* carrying aphids were observed to be dropped off the host plant before sporulation, possibly explaining the fact on reduction of risk of infection. So how do these microbes sense the danger? Does any kind of cross-talk exist? And is it the presence of the fungus or the sensing mechanism of the microbes that is the major player here? Such studies definitely require us to bring the fungus, in question, into focus.

Body colour

The secondary symbiont *Rickettsiella* changes the body colour notably from red to green due to increase in blue green polycyclic quinones in natural populations of pea aphids[21]. It seems that the combination of aphid genotype and endosymbionts contribute towards the host body colour. Thus, this opens an avenue for working towards the ecologically important traits involved in species recognition, predation, parasitism, sexual selection, mimicry, crypsis and aposematism.

Reproduction

Aphids reproduce by parthenogenesis which is cyclic in nature with alternate clonal and sexual reproduction annually. Investigation reveals that many secondary symbionts exhibited detrimental effects under sex inducing conditions[22]. Their effect depends on the genotype of pea aphid. Plus, *Rickettsia* exhibited an influence on longevity and fecundity of asexual genotypes. *Spiroplasma* exhibited a negative impact on host fitness. It has not been clear yet if the facultative symbionts play any strong role in asexual cycle and in mating. However, ibpA genes in *Buchnera* show some kind of effect but only in response to the environmental temperature.

Host Plant Selection

An evolutionarily important trait for their growth and survival is host plant selection. This was attributed to the presence of facultative endosymbiotic bacteria, in addition to the aphid genotype when the effect of facultative symbionts on aphids for host plant specialization was examined[23]. On injecting *H. defensa* to uninfected pea aphid clones fecundity of the recipients was reduced. However, co-infection with *Spiroplasma* or *Rickettsia* appeared to have a slightly less severe impact. Therefore no proper evidence was found regarding the influence of secondary symbionts on host plant specialization. For examining the influence of *R. insecticola* on pea aphid's choice of host plants using *Vicia fabae* and *Trifolium pratense,* five isolates of the species were injected into the host to look for any variation. Only one showed enhanced performance on *T. pratense*[24]. The primary symbiont *Buchnera aphidicola* contributes towards survival on a nutritionally imbalanced diet of phloem sap by synthesizing amino acids deficient in aphid diet[25]. Further, proteins encoded by *Buchnera* and *Rickettsia* had an impact on gaining nutrition from resistant plants which the avirulent aphids were unable to derive[26]. There lies a possibility here for the secondary symbionts to hold any influence on the primary symbiont or vice versa.

Table-1
Endosymbionts that provide various benefits to the aphids

Bacteria	Host aphid	Nature of benefit	Researchers involved
Buchnera aphidicola	*Acyrthosiphon pisum*	Nutrition, fecundity	Moran & Degnan (2005)
Serratia symbiotica	*Acyrthosiphon pisum*	Heat tolerance	Burke G et al (2010)
Regiella insecticola	*Myzus persicae*	Resistance against parasitoid attack	Vorburger et al (2009)
Hamiltonella defensa	*Acyrthosiphon pisum*	Resistance against parasitoid attack	Oliver et al (2005, 2009) Costopoulos et al (2014)
Serratia symbiotica	*Acyrthosiphon pisum*	Resistance against parasitoid attack	Oliver et al (2005); Costopoulos et al (2014)
Hamiltonella defensa	*Aphis fabae*	Resistance against parasitoid attack	Cayetano et al (2013)
Hamiltonella defensa	*Sitobion avenae*	Resistance against parasitoid attack	Sajjad Ali (2015)
Regiella insecticola	*Acyrthosiphon pisum*	Resistance against Fungus	Scarborough et al (2005) Parker et al (2013) Lukasik et al (2013)
Rickettsiella	*Acyrthosiphon pisum*	Resistance against Fungus	Godfray et al (2013)
Ricketsiella	*Acyrthosiphon pisum*	Change in body colour	Tsuchida et al(2010)
Rickettsia	*Acyrthosiphon pisum*	Reproduction	Simon et al (2011)
Regiella insecticola	*Acyrthosiphon pisum*	Host plant specialization	Ferrari et al (2007)
Buchnera aphidicola	*Acyrthosiphon pisum*	Host plant specialization	Douglas et al (2006)
Rickettsia	*Acyrthosiphon pisum*	Host plant specialization	Francis et al (2010)

Variation in the presence of bacterial strains

Aphids infecting particular family of plants vary in the composition, abundance and strains of the bacterial species they harbour. The bacteria dominant in a specific aphid may be patchily present in another due to geographical reasons. The diversity of their microbial flora can be documented by molecular profiling. In a particular study on bacterial diversity of North American pea aphids multiple strains of the bacteria *R. insecticola, H. defensa*, and *Rickettsia* were detected[27]. Their frequency varied with location and host plant utilization of aphids. Usually, a combination of Denaturing Gradient Gel Electrophoresis (DGGE) and pyro sequencing followed by 16S rRNA, housekeeping and symbiont associated bacteriophage genes are examined for such studies. The symbionts differ in their ability and number to defend their hosts against their natural enemies along with the varying strains, aphid hosts, locations etc. For example, two strains of *Regiella insecticola* appeared to differ in their ability of protecting the pea aphids against the parasitoid *A. ervi.* The strain *R. insecticola* 5.15 increased aphid survivorship against the parasitoid which *R. insecticola* LSR1 failed to do. The difference in defense mechanism owes to the presence of few pathogenicity factors in the former that are either inactivated or lacking in the latter strain[28]. *Buchnera* APS of pea aphid and *Buchnera* Mp of green peach aphid differ in their genomic content by 21 additional stains in the latter[29]. Similarly, *Buchnera* of the aphids *A. pisum* and *S. graminum* show divergence 50-70 million years ago. In a study where the bean aphids harbouring different isolates of *H. defensa* were exposed to the parasitoid, it was found out that they suffered varying level of parasitism owing to the different isolates of bacteria[30]. Degnan et al (2010) had almost sequenced the *R.insecticola* genome and compared with that of *H. defensa*, which too, had been sequenced.

Genetic basis of the conferred benefits

Beside the nuclear and organellar transmission cytoplasmic transmission are likely to take place in bacterial symbionts of aphids. In case of the nutritional benefit provisioning *Buchnera,* the genes trpE and trpG encode for anthranilate synthase, a rate limiting enzyme in tryptophan biosynthetic pathway. Phylogenetic analysis of the congruent trees in the genes of the aphid host mitochondria and the bacteria have confirmed the fact of the vertical transmission of the bacteria[10] . Also, leuABCD encoding enzymes for leucine biosynthesis that were reported in the plasmids of aphids *Schizaphis graminum* and *Diuraphis noxia* had a close resemblance with the plasmid of *Buchnera* of *Rhopalosiphum padi*[31]. Again, one of the two strains of the species *R. insecticola* has been found to possess additional genes for encoding five pathogenicity factors unlike for the other strain[28]. Ishikawa et al examined that the genome copy number of *Buchnera* at first increased from aphid embryonic stage to adulthood and then decreased with age[31]. Density of the facultative symbionts is influenced by the presence of other symbionts as in case of pea aphid where the density of *S. symbiotica* increases during superinfection of the hosts in a study conducted by Oliver et al[32].

Conclusion

While there are bacterial strains that show negative effects in terms of the longevity, reproduction, survival there are some which although present, are reported to exhibit no effects at all. *Arsenophonus* can be cited as an example for such case[33]. *Arsenophonus* harboured by *Aphis glycines* has shown no defensive effect against natural enemies.

Not all the strains should show the similar patterns of defensive property against parasites. Strains, geographical locations, climatic conditions and the microclimatic conditions too must be taken into account to study the pattern in their variation. Also the host plant to which the aphid is restricted to, possibly plays a role in functioning of the symbionts[34].

A latest report[35] says that a protein GroEL originating from *Buchnera aphidicola* induces plant immune response against the aphid harbouring the bacteria. This fact brings up questions related to the various costs that aphids have to incur for harbouring the *Buchnera* strains and further how do they vary across aphid and plant species? The same question applies when non- synonymous mutations and population bottlenecks drive groEL evolution in *Buchnera*[36].

The symbionts exhibit interspecies host transmission whose mechanism is unknown. We must try to find out the sync among the plant-aphid-endosymbionts and their coevolutionary dynamics.

Although an attempt has been made in the past to draw a molecular phylogeny of the aphids based on the symbionts they harbour, would it be valid to consider the metabolic pathways (i. e. chemotaxonomy) solely for the phylogenetic analysis?

Do the endosymbionts possess any influence on the wing formation as well as flight of aphids? Addressing this question would shed light on the mutualism of the aphid- bacteria from newer perspectives to understand their evolutionary ecology.

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