ISJ 8: 247-255, 2011 ISSN 1824-307X

REVIEW

The evolutionary ecology of aphids' immunity

M Poirié[1,2,3], C Coustau[1,2,3]

[1]*From the Evolution and Specificity of Multitrophic Interactions (ESIM), UMR 1301 "Biotic Interactions and Plant Health" (IBSV), Institut National de la Recherche Agronomique, INRA PACA, Sophia Antipolis, France*
[2] *UMR 6243, Centre National de la Recherche Scientifique, CNRS, France*
[3] *Université Nice Sophia Antipolis, UFR Sciences, France*

Accepted December 19, 2011

Abstract

Aphids comprise 4,400 species that live in close interactions with their host-plants, the parasitoid wasps and fungi they encounter, as well as several bacteria including *Buchnera aphidicola*, an obligatory, nutrient-providing symbiont. Aphids also interact with a cohort of facultative secondary symbionts that strongly interfere with their major life history traits such as host-plant specialization, heat tolerance and resistance to natural enemies. Here, we present some evolutionary and ecologically-relevant aspects of these interactions, focusing on aphid defenses to parasitism, and considering aphids either as "extended organisms" comprising aphid and symbionts' genomes, or as "single-genome" organisms whose immune components are still poorly known. We highlight the complexity of predicting evolution of aphid immune resistance in the field, due to variable selection pressures, short-term costs, and cross-talk between symbionts. Finally, we present perspectives to strongly improve our understanding of the "aphid-symbiont-bacteriophage" meta-organism defenses and to elucidate the interactions between immunity, pathogenicity and symbiosis.

Key Words: aphid; immune defenses; symbionts; parasitoids; extended phenotype, ecological immunity

Introduction

All organisms have to maintain homeostasis and ensure growth and reproduction in changing abiotic and biotic environments. There is no doubt that one of the most challenging environmental condition is the presence of a large diversity of pathogens and parasites, and that the main host defense relies on the immune system. Furthermore, the existence of a continuum from pathogenic to beneficial microorganisms is now largely admitted and current research focuses on the immune system as a main factor in the establishment and maintenance of mutualist/symbiotic interactions (Slack *et al.*, 2009; Login *et al.*, 2011). Investigation of the complex interactions between immunity, pathogenicity and symbiosis mostly relies on insect models that are numerous and diversified. Indeed, it is roughly estimated that more than 70 % of species host one or more bacterial symbionts (Hurst and Darby, 2009).

Corresponding author:
Marylène Poirié
Evolution and Specificity of Multitrophic Interactions (ESIM)
UMR 1301 "Biotic Interactions and Plant Health" (IBSV)
Institut National de la Recherche Agronomique INRA PACA,
400 route des Chappes, Sophia Antipolis, 06903, France
E-mail:marylene.poirie@sophia.inra.fr

The complex pathways of innate immunity have been at least partly deciphered in model species, allowing comparative analyses to be performed. Of course, as immunity is a major fitness-related trait, interest is also given to variation of some immune components in relation with biological characteristics such as the developmental stage, the morph, the sex, or the occurrence of a previous immune challenge, as well as with physical environmental characteristics, such as the temperature. Evolutionary important features as the existence of trade-offs between constitutive or induced immunity and, for instance, survival or reproduction, are also considered, and the evolution of the level of specificity of the immune response is largely discussed (Sadd and Schmidt-Hempel, 2009; Schulenburg *et al.*, 2009). However, data on all these aspects are scarce in Homopteran insects and notably in one of the most representative groups, the aphids.

Aphids are remarkable organisms at the evolutionary and ecological level that have adapted to drastic nutritional and ecological constraints thanks to specific life-history traits and complex polymorphisms. Firstly, their life-cycle is characterized by a succession of sexual and asexual morphs, dependent on the environmental

conditions. Their impressive ability to proliferate thus largely relies on clonal multiplication while the resulting lack of genetic diversity is mainly compensated by a high phenotypic plasticity. Dissemination and colonization of new host plants is ensured by the production of winged individuals when resources become scarce (Le Ralec *et al.*, 2010).

Secondly, the biology of aphids is characterized by multiple inter-specific interactions. They are sap-feeding insects that establish a durable interaction with their host plant, managing to avoid or control the plant defenses, and manipulating the plant physiology to ensure the compatibility of the interaction (Giordanengo *et al.*, 2010). Adaptation to the restricted phloem sieve diet is ensured by an obligate (primary) symbiosis with the bacteria *Buchnera aphidicola* which is essential in providing the missing nutrients (amino acids) (Brinza *et al.*, 2009). Aphids can also carry secondary, facultative symbionts that are not required for survival but can be mutualistic in affecting positively various life history traits such as suitability to the plant host, heat tolerance, or protection against natural enemies (Montlor *et al.*, 2002; Oliver *et al.*, 2010). Finally, like all living organisms, aphids are attacked by natural enemies such as pathogenic fungi and parasitoid wasps.

The immunity of aphids is of particularly interest as it likely affects the network of inter-specific interactions, therefore playing a central role in their ecology and evolution (Fig. 1). Understanding the functioning and evolutionary ecology of aphid immune defenses is therefore of central importance for future development of control strategies involving pathogenic agents or targeting the aphid-symbionts equilibrium. However, data in this area remain scarce. Here, we present a brief overview of our current knowledge, mainly focusing on the pea aphid *A. pisum* whose genome has been sequenced, and which represents a good example of aphids' functioning as a meta-organism. We then discuss the evolutionary and ecologically-relevant traits of aphid biology that should be explored in the next future in relation to immune findings.

The aphid complex biology

Aphid diversity and plasticity

Aphids belong to the *Aphidoidea* and the *Phylloxeroidea* super families of Hemiptera and they comprises about 4,400 species (Blackman and Eastop, 1994). Among these, about 250 species are agricultural pests, mainly because they vehicle and transmit plant viruses. Most aphid species are found in temperate regions but some have adapted to tropical environments (Dixon *et al.*, 1986) or even extreme climates such as sub-Antarctic (Hullé *et al.*, 2003), resulting in a world-wide distribution. Aphids can feed on virtually all plant families, the majority of species being specialized to a single host plant, while some have a broad host-plant range (Pecoud *et al.*, 2010). Aphid speciation and diversification is thought to be widely driven by their specific adaptation to host plants (Pecoud *et al.*, 2010).

Aphid life cycles involve a succession of sexual and asexual morphs. In the simplest cycles, such as that of the pea aphid *Acyrthosiphon pisum*, a single sexual generation occurring in autumn alternates with several parthenogenetic generations where each female produces hundreds of viviparous offspring (Helle, 1987). Changes in sexual fate and reproductive mode are condition-dependent and they illustrate the aphid extraordinary developmental plasticity in response to environmental cues. Altogether, whether variability of a given trait of aphids results from an existing genetic diversity among clones, as evidenced for their adaptation to the host plant, or from high phenotypic plasticity, is sometimes difficult to establish.

Aphid multiple inter-specific interactions

One of the major characteristics of nearly all aphids is their adaptation to plant feeding through association with the obligatory nutrient-providing bacterial symbiont *Buchnera aphidicola*. This Gram-negative proteobacterium has co-evolved with aphids for 160-280 millions years (Moran and Baumann, 1994; Wilson *et al.*, 2010). Bacteria are located only in specialized cells, the bacteriocytes, and they are transmitted vertically to the embryos. *Buchnera* has a dramatically reduced genome (<1Mb), typical of well-integrated obligatory intracellular endosymbionts, where genetic and metabolic redundancy has been minimized (Gil *et al.*, 2002; Toft and Andersson, 2010). It has been estimated that nearly 10 % of the coding capacity is devoted to biosynthesis of 10 essential amino acids that are lacking in the aphid phloem sap diet (Wilson *et al.*, 2010). While metabolic interactions between aphids and *Buchnera* have been extensively studied (Hansen and Moran, 2011), the role of the aphid immune system in the establishment and maintenance of this mutualistic interaction remains to be examined.

Aphids are attacked by various enemies, notably parasitoid wasps. Primary parasitoids of aphids belong to two specialized taxa, the sub-family *Aphidiinae* (Hymenoptera: *Braconidae*) and the genus Aphelinus (Hymenoptera: *Aphelinidae*). Female wasps lay eggs in different developmental stages of aphids, from larvae to adults. By the time the parasitoid larvae is fully developed, the aphid dies and its cuticle hardens to form a so-called "mummy" from which an adult wasp will emerge (Le Ralec *et al.*, 2010). Aphids are also infected by various fungi, which generally induce death within a few days (Butt *et al.*, 1990). Differences in aphid susceptibility/resistance to parasitoids or pathogens have been reported in the field (Henter and Via, 1995) but the underlying mechanisms are still largely unknown.

Finally, aphids also interact with bacterial secondary endosymbionts (Oliver *et al.*, 2010). They are facultative and found free in the hemolymph as well as within various cell types including bacteriocytes (Oliver *et al.*, 2010). Interestingly, secondary symbionts can impact important fitness-related traits, further complicating the evolutionary ecology of aphids. For instance, *Serratia symbiotica* has a beneficial effect on *A. pisum* reproduction and viability under heat stress (Montllor *et al.*, 2002), thus providing a functional explanation to the previous observations that its frequency reached 80

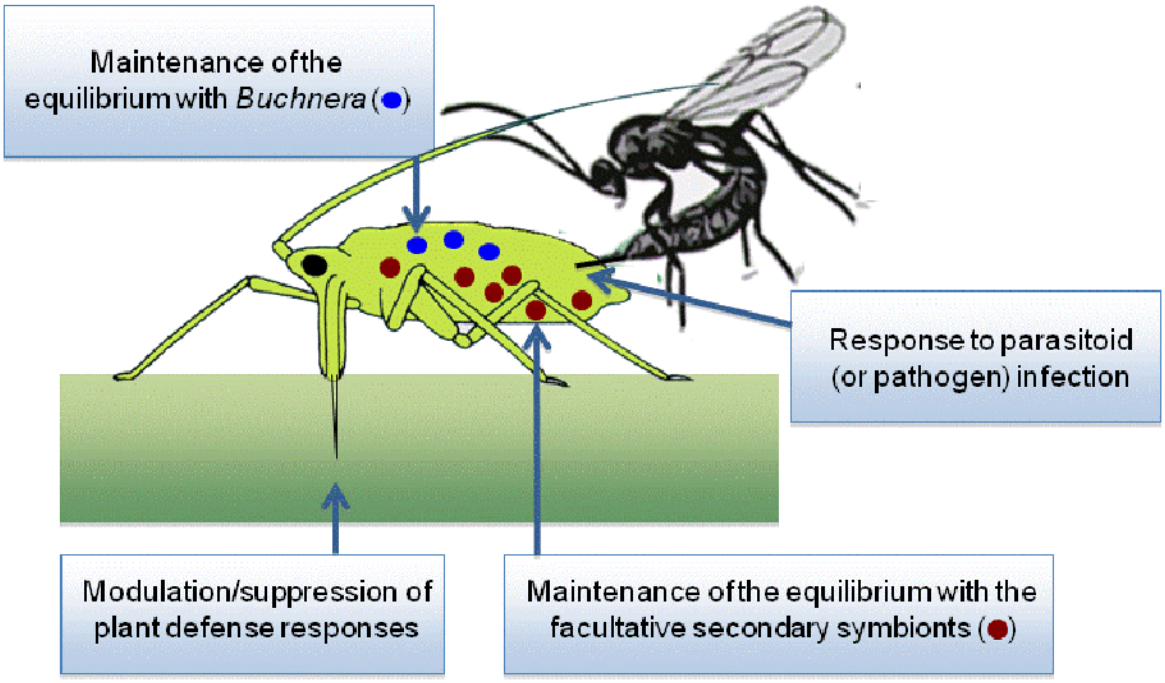

Fig. 1 Aphid's immunity is likely involved in interactions with the host plant, the primary and secondary symbionts, as well as the pathogens or parasitoids.

% in hot places (Oliver *et al.*, 2010). Another reported symbiont effect is the change in aphid color. A recent study indeed evidenced that the presence of *Rickettsiella* induces a body color change from red to green (Tsuchida *et al.,* 2010). Such a modification is likely to affect prey-predator-parasite interactions since ladybird beetles preferentially consume red aphids while parasitoids are more attracted by green ones. Finally, a largely affected fitness-related trait is adaptation to the host plant. In particular, results from several independent studies revealed a complex association between infection by *Regiella insecticola*, the aphid genotype, and the host plant use (Ferrari *et al.*, 2007). Host-plant specialization of aphids can also be directly affected, as infection by *R. insecticola* would improve aphids' fitness specifically on clover (Tsuchida *et al.*, 2004).

Most fitness-related traits and interactions of aphids with other species can therefore be diversely affected by the presence of symbionts, whether alone or in combination (Oliver *et al.*, 2006).

Symbiont transmission

In contrast to *B. aphidicola*, secondary symbionts are generally transmitted vertically. However, occasional horizontal transmission has been reported. For instance, one secondary symbiont was shown to be possibly transmitted through artificial diet, and its presence was reported in aphid honeydew as well as siphuncular fluid samples (Darby and Douglas, 2003). The lateral transfer of symbionts may not only generate exchanges between otherwise independent clonal lines but also allow a much quicker spread of symbionts among populations. Most interestingly, symbiont transmission was also reported to differ between the parthenogenetic and sexual reproduction stages. First, the maternal transfer of symbionts appeared to be far more imperfect during sexual reproduction than during parthenogenesis, which might be a source of uninfected aphids (Moran and Dunbar, 2006). Besides, paternal transfer of symbionts could lead either to infection of previously non-infected aphids, to double infections, or to replacement of the maternal symbiont (Moran and Dunbar, 2006). The occurrence of paternal transfer of symbionts likely impacts their population dynamics, notably because of the possible establishment of double infections. New combinations of symbionts might indeed confer new characteristics to the host, as well as generate synergistic or antagonistic interactions. In addition, recombination events and phage gene exchanges might occur, representing a source for rapid evolutionary changes.

The evolutionary ecology of aphids immune interactions

One major difficulty in understanding how ecological factors, either biotic or abiotic, shape the evolution of the immune system is probably that this question - that defines ecological immunology (Schulenburg, 2009) - is at the interface between different scientific areas. As secondary symbionts are main components of the biotic environment of aphids and strongly influence their resistance to pathogens, the study of aphid defenses belongs naturally to ecological immunity. The use of classical tools to estimate overall defenses (survival to pathogens, encapsulation ability, hemocyte numbers, phagocytic activity, phenol oxidase activity, antimicrobial activity, quantitative PCR on

immune-relevant genes, etc.) under various biotic and abiotic conditions is thus a major approach to explain and predict the complex interactions between symbiosis and immunity in the aphid model.

An important feature at this time is also the definition of the "organism" to be considered. Aphids can indeed be perceived either as species whose immune phenotype is mainly determined by their own genome, or as "extended organisms"[1] (in the sense of Dawkins' extended phenotype) or meta-organisms, whose defenses may result from the intricate effect of different genomes, including that of symbionts.

Aphids as "extended organisms"

Though the aphid meta-organism was reported to interact with host-plants, parasitoids and pathogenic fungi, studies have mainly focused on the "resistance to parasitoids" phenotype, and more specifically on the resistance associated with secondary symbionts.

Clonal resistance to braconid parasitoids has been described in populations of *A. pisum* (Hufbauer and Via, 1999; Ferrari *et al.*, 2001), *Myzus persicae* (von Burg *et al.*, 2008) and *Aphis fabae* (Vorburger *et al.*, 2009), although it is quite rare. In a resistant aphid, failure of the parasitoid can occur either at an early stage when the egg fails to develop or at the larval stage (Li *et al.*, 2002), and it is the "larval stage" resistance that is largely influenced by secondary symbionts. In order to understand how symbionts increase aphids' resistance, several studies have experimentally manipulated the symbiotic associations, either by suppressing symbionts using antibiotics treatment, or by introducing a new symbiont thanks to micro-injection. For instance, *Aphidius ervi* parasitism success on *A. pisum* was shown to be reduced by 42 % and 23 % in aphid lines harboring *H. defensa* and *S. symbiotica* respectively (Oliver *et al.*, 2003). When aphids were experimentally super-infected with both symbionts, the reduction in parasitism success reached 60 %, a benefit that correlated with a marked decrease in fecundity (Oliver *et al.*, 2006). *H. defensa* was also reported to provide resistance to *A. ervi* against *Aphidius eadyi* (Ferrari *et al.*, 2004; Oliver *et al.*, 2009), and more recently to *Aphis fabae* against *Lysiphlebus fabarum* (Vorburger *et al.*, 2009).

It is noteworthy that different strains of *H. defensa* confer various levels of protection against *A. ervi* (Oliver *et al.*, 2005). Strikingly, however, a toxin-encoding bacteriophage, APSE (*A. pisum* secondary endosymbiont), was demonstrated to be required, and likely responsible, for the protective phenotype (Oliver *et al.,* 2009). More recently, an independent study evidenced that *A. pisum* clones infected with both *H. defensa* and the newly discovered symbiont PAXS displayed a high resistance to *A. ervi* (Guay *et al.*, 2009). Another symbiont, *Regiella insecticola,* was previously known to confer resistance to a fungal pathogen (Scarborough *et al.*, 2005) but not to parasitoids. However, unlike other strains of this bacterium, a specific isolate from *Myzus persicae* provides a protection against the wasp *Aphidius colemani* (Vorburger *et al.*, 2010).

Altogether, these data suggests that the ability to protect the host against natural enemies may evolve readily in different endosymbiotic bacteria, maybe in relation with occurrence of genetic exchanges and gene transfer among symbionts or phages in double-infected hosts (see above). To date, the only described mechanism explaining the symbiont-associated protection is a direct effect, involving the use of bacteriophage' toxins. However, bacterial toxins might be used as well, since most symbionts, including Hamiltonella have retained virulence-associated genes in their genomes (Degnan *et al.*, 2009). Alternatively, symbionts may act indirectly, through an existing host system, and, for instance, positively affect the host immunity.

The evolution of symbiont-associated resistance in populations depends on the selection pressures induced by parasitism rates, on the costs associated with the presence of a given symbiont and of the cross-talk between the symbiotic companions in case of multi-infection (Oliver *et al.*, 2006). Interestingly, the symbiont-associated cost may itself vary. Most facultative symbionts have detrimental effects on their host fitness under sex-inducing conditions (Simon *et al.*, 2011), and the estimated cost on *Aphis fabae* longevity associated with Hamiltonella depends on genotype×genotype interactions between the host and the symbiont (Vorburger and Gouskov, 2011). The selective advantage provided by symbionts can also vary. For instance, the resistance associated with Hamiltonella's bacteriophage evolves quickly due to repeated losses of the phage under laboratory conditions, probably because of an imperfect vertical transmission (Oliver *et al.*, 2009). Resistance to *A. ervi* in the presence of *H. defensa* can also change from complete protection to high susceptibility depending on the temperature (Bensadia *et al.*, 2006). Finally, parasitoids exposed to *H. defensa*-harboring resistant clones rapidly gain virulence over time (Dion *et al.*, 2011), so that resistance is overcome, but they also experience a reduction in fitness.

The case of *Hamiltonella* well illustrates the prediction that symbiont-associated resistance may be less stable than genetic resistance (Hurst and Darby, 2009). Indeed, the rate of vertical transmission is not always 100 %, so that bacteria can be lost, and the presence of symbionts is possibly costly, at least energetically. The complex pattern of selective advantages and disadvantages may then explain the large fluctuations of *Hamiltonella* frequency and aphid resistance to parasitoids reported in the field. Also, it may facilitate the acquisition/evolution of new resistance-associated secondary symbionts (maybe explaining the observed resistance conferred by PAXS in association with *Hamiltonella,* Guay *et al.*, 2009).

[1] The term "extended-species" we initially choose do not strictly apply to the aphid model since the host and the symbionts, including Buchnera, are considered as different taxonomic units. However, we have clear examples from the long-term evolution that an intricate symbiosis can ultimately lead to the formation of a unique taxonomic entity.

Aphids as a "single genome" species

Vorburger *et al.* (2008) has suggested that aphid parasitoids may be confronted with two lines of defense: the "innate defences" and the "acquired defences" provided by secondary endosymbionts, which likely differ in their effectiveness and specificity. Significant clonal variation in resistance was indeed observed in several studies, which suggest the existence of an aphid innate resistance. For example, susceptibility of the pea aphid to *A. ervi* was shown to vary among clones of a single population (Henter and Via, 1995). Although occurrence of such a "genetic" variation suggested a potential for resistance to evolve in response to selection, the average resistance remained unchanged between aphids from this population collected early or late in the summer and exposed meanwhile to a high parasitism rate (Henter and Via, 1995). The authors hypothesized that the lack of response to selection was due to trade-offs between resistance and other fitness-related traits. Significant clonal variation and co-variation in resistance of *A. pisum* to two parasitoid wasps and to a pathogenic fungi was also reported, without evidence of a trade-off between resistance and fecundity (Ferrari *et al.*, 2001). On the contrary, *Myzus persicae* effectiveness to survive *A. colemani* attacks was correlated with a loss of fecundity in individuals surviving the attack (Vorburger *et al.*, 2008). In other words, clones that were more resistant to the parasitoid experienced a higher loss in fecundity when attacked. Such a trade-off may impair selection for resistance in natural populations and participate to the maintenance of genetic variation for resistance (Vorburger *et al.*, 2008). Finally, recent work from Dion *et al.* (2011) also demonstrated a large clonal variation in resistance to *A. ervi* in the absence of secondary symbionts.

Importantly, caution must be taken in concluding on the genetic basis of a variation in resistance since the absence of secondary symbionts has not always been assessed or accurately demonstrated. When tested, the presence of symbionts was assessed through PCR analysis based on known sequences, while novel aphid secondary symbionts are regularly described (Guay *et al.*, 2009; Tsushida *et al.*, 2010).

Although these studies nevertheless highlight natural variation in aphid ability to fight pathogens, the mechanisms underlying this variation are totally unknown and the involvement of the immune system has not been investigated. Understanding resistance to parasitoids in aphids and their evolution thus requires a thorough study of aphids' own immune defences, as well as of the parasitoid strategies selected to avoid or circumvent all aphid defense categories.

The aphid immune system: what do we know?

Intriguingly, neither the physiology nor the molecular biology of the immune defenses of aphids have ever attracted attention. Possible reasons for that are the small size of most aphid species. Also, insect immunity was primary studied on Diptera and Lepidoptera that are submitted to frequent bacterial challenges, while aphids belong to the Homoptera and were mainly described as interacting with parasitoid wasps. Ecological immunologists often estimate the immune response level by counting the number of immune cells, and measuring the phenol oxidase (PO) activity. However, there are very few available descriptions of immune cells in aphids, the most detailed being by far the one of Boiteau and Perron (1976), which described six hemocyte categories in *Macrosiphon euphorbiae*: prohemocytes, oenocytoids, plasmatocytes, granulocytes, spherulocytes and wax cells. Surprisingly, the first data on *A. pisum* (Laughton *et al.*, 2011), reported only three morphologically distinct types of hemocytes: prohemocytes, granulocytes that may phagocyte bacteria, and oenocytoids. More accurate, thorough analyses, including ultra-structural description of the cells, and description of their adhesion profiles, are strongly needed to perform functional analyses. Comparison of aphid hemocyte numbers from different morphs and under different environmental conditions nevertheless remains a complicated task, due to the low cell number and the quantity of debris and symbionts in the hemolymph. Regarding the phenoloxidase pathway, detailed annotation work of *A. pisum* genome suggests that it exists in the pea aphid (Gerardo *et al.*, 2010), and a constitutive phenoloxidase activity can be measured (M Poirié, personal data). Whether or not it differs between morphs and can be activated by a pathogen challenge remains to be established.

The question of the immune molecular processes underlying the biotic interactions of aphids is then far from being elucidated. Most information comes from the recent sequencing of the first aphid genome (*A. pisum*) by the International Aphid Genomics Consortium (IAGC) (IAGC, 2010) that has raised novel evolutionarily- and functionally-relevant questions. For instance, a total of approximately 34,000 genes were predicted, which is nearly twice as described for other insect sequenced genomes belonging to *Diptera, Coleoptera* and *Hymenoptera* (IAGC, 2010; Tagu *et al.*, 2010). This is at least partly explained by the existence of many gene duplications (Tagu *et al.*, 2010).

In a first search for immune-related genes in *A. pisum* genome, Gerardo and collaborators (2010) identified key elements of the Toll and Janus kinase/signal transducer (JAK/STAT) pathways, as well as corresponding recognition and effector genes. Surprisingly, however, the immune deficiency (IMD) signaling pathway was apparently non functional, with some of the genes missing, and no peptidoglycan recognition proteins (PGRPs) were found. In addition, well-conserved antimicrobial peptides such as defensins and cecropins could not be predicted (Gerardo *et al.*, 2010). Experimental analyses were designed to characterize immune response through the isolation of RNA transcripts from immune-challenged pea aphids but they uncovered few immune-related products. These data and the low expression levels of some characterized aphid immune genes suggested a low overall antibacterial immune response (Altincicek, 2008; Gerardo, 2010) in agreement with aphid susceptibility to experimental bacterial infection (Grenier, 2006; Altincicek, 2011).

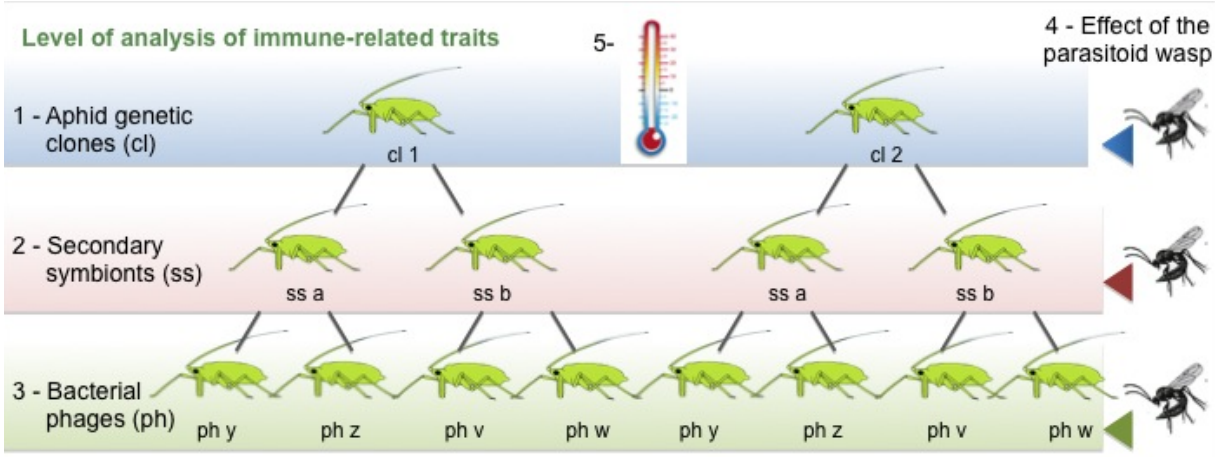

Fig. 2 Studies on aphid immune-related traits should be performed in a sequential manner aimed at understanding the respective influence of aphid genotype (level 1), of the presence of secondary symbionts (level 2), and possibly the presence of phages in secondary symbionts (level 3). The parasitoid effect should be tested in combination with all these levels and the temperature effect will likely have to be considered as well. Note that secondary symbionts may belong to different species or may represent different strains of a given species, therefore complexifying the approach.

Different evolutionary hypotheses have been proposed to explain this surprising result. For instance, aphid increased investment in reproduction following infection, or symbiont-mediated host protection might "compensate" for the "deficient" immunity. This latter hypothesis implies of course that symbionts do not act indirectly through manipulation of host immunity. Also, the reduced antibacterial defense was suggested to be an adaptation for the symbiosis with the bacterium *Buchnera aphidicola*, that is known to elicit an immune response in *Drosophila* S2 cells (Douglas *et al.*, 2011). This selection to "accommodate" the bacterial partner could have also ended in a reduced antibacterial defense specific to the bacteriome as reported in a weewil species (Anselme *et al.*, 2008) given that *Buchnera* cells are rarely encountered outside the bacteriome.

A main concern in answering the question of a “deficient” or a “different” immune system in aphids is the lack of information both on genes potentially involved in the anti-parasitoid response, and on occurrence of resistance to bacterial pathogens. Besides, it is possible that a substantial part of the aphid immune genes escaped annotation due either to assembly problems or to biases, since gene prediction and identification strictly rely on similarities with genes previously described in other models. The "deficient immunity" hypothesis thus remains first to be tested accurately, taking into account other elements of the immune response such as the signaling pathways involved in cellular responses (including MAPK pathways), the receptors involved in phagocytosis ability, or the reactive oxygen species-mediated defenses.

Future directions

Aphid’s life history traits, including immune performances, must be viewed as extended phenotypes (Dawkins, 1989) resulting not only from the expression of the aphid genome itself, but also from the expression of genes from their bacterial symbionts and eventually from the bacterial phages. In many insects, including *Drosophila,* bacterial symbionts can indeed positively or negatively impact host defenses against pathogens and even participate in the formation of the immune system (Xie *et al.*, 2010; Weiss *et al.*, 2011). Characterization of immune traits thus have to be performed in aphids naturally or artificially deprived of secondary symbionts. This will allow subsequent comparison of different genetic backgrounds and different morphs, under different conditions (Fig. 2). In a second step, it will be possible to compare immune components between genetically identical lines without secondary symbionts or with a single secondary symbiont, or different strains of this symbiont, with or without associated phages (Fig. 2). This will provide essential information on how the aphid immune system and the symbionts interfere with each other, depending of the symbiont strain or species. Strikingly, understanding the immune ecology of aphids as meta-organisms will also require addressing the important question of the multiple-infections and of the impact of abiotic conditions.

In the field, future studies aimed at characterizing immune processes or at examining an immune-related trait, such as the ability to fight infection by a particular pathogen or parasitoid, should be carefully designed to control or characterize the extended genotype (Fig. 2). The diagnostic of the presence of microorganisms by observatory methods (such as microscopy, immune-labeling or PCR) is restricted to the known symbiont species. However, the rapid progression of genome-sequencing methods and facilities should allow characterization of the metagenome of aphid clones in a close future, opening the way to comparative

genomics of clones presenting different immune-related traits (*i.e.*, resistance/susceptibility to a pathogen).

Applied to human gut, microbial metagenomics recently revealed more than 1,000 prevalent bacterial species in a single cohort of 124 individuals, each individual hosting at least 160 of such species (Qin *et al.*, 2010), whether commensal or potentially pathogenic. This commensal microbiota is now known to shape the host immune system. Aphids host a comparatively much smaller number of bacteria but they have highly intricate relationships with most of them, then appearing as good models for deciphering the interactions between immunity, pathogenicity, and symbiosis. They are also important models to address the central question of how to use our increasing knowledge on the symbiont-mediated modification of essential life-history traits for improving human and plant health.

Acknowledgments

We are thankful to JC Simon, JL Gatti and A Schmitz for fruitful discussions as well as to an anonymous reviewer for comments and suggestions to improve the manuscript.

References

Altincicek B, Gross J, Vilcinskas A. Wounding-mediated gene expression and accelerated viviparous reproduction of the pea aphid *Acyrthosiphon pisum*. Insect Mol. Biol. 17: 711-716, 2008.

Altincicek B, Ter Braak B, Laughton AM, Udekwu KI, Gerardo NM. *Escherichia coli* K-12 pathogenicity in the pea aphid, *Acyrthosiphon pisum,* reveals reduced antibacterial defense in aphids. Dev. Comp. Immunol. 35: 1091-1097, 2011.

Anselme C, Pérez-Brocal V, Vallier A, Vincent-Monegat C, Charif D, Latorre A, Moya A, Heddi A. Identification of the weevil immune genes and their expression in the bacteriome tissue. BMC Biol. 6:43, 2008.

Bensadia F, Boudreault S, Guay JF, Michaud D, Cloutier C. Aphid clonal resistance to a parasitoid fails under heat stress. J. Insect Physiol. 52: 146-157, 2006.

Blackman RL, Eastop VF. Aphids on the world's trees. CAB International, Wallingford, United Kingdom, 2004.

Boiteau G, Perron J.-M. Etude des hémocytes de *Macrosiphum euphorbiae* (Thomas) (Homoptera: Aphididae). Can. J. Zool. 54: 228-234, 1976.

Brinza L, Viñuelas J, Cottret L, Calevro F, Rahbé Y, Febvay G, *et al*. Systemic analysis of the symbiotic function of *Buchnera aphidicola*, the primary endosymbiont of the pea aphid *Acyrthosiphon pisum*. CR Biol. 332: 1034-1049, 2009.

Butt TM, Becket A, Wilding N. A histological study of the invasive and developmental processes of the aphid pathogen *Erynia neoaphidis* (Zygomycotina: Entomophthorales) in the pea aphid *Acyrthosiphon pisum.* Can. J. Bot. 68: 2153-2163, 1990.

Darby AC, Douglas AE. Elucidation of the transmission patterns of an insect-borne bacterium. Appl. Envir. Microbiol. 69: 4403-4407, 2003.

Dawkins R. The Extended Phenotype. Oxford University Press, Oxford, 1989.

Degnan PH, Yu Y, Sisneros N, Wing RA, Moran NA. *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. Proc. Natl. Acad. Sci. USA 106: 9063-9068, 2009.

Dion E, Zélé F, Simon JC, Outreman Y. Rapid evolution of parasitoids when faced with the symbiont-mediated resistance of their hosts. J. Evol. Biol. 24: 741-750, 2011.

Dixon AFG, Kindlmann P, Leps J, Holman J. Why there are so few species of aphids, especially in the tropics. Am. Nat. 129: 580-592, 1986.

Douglas AE, Bouvaine S, Russell RR. How the insect immune system interacts with an obligate symbiotic bacterium. Proc. Biol. Sci. 278: 333-338, 2011.

Ferrari J, Muller CB, Kraaijveld AR, Godfray HCJ. Clonal variation and covariation in aphid resistance to parasitoids and pathogen. Evolution 9: 1805-1814, 2001.

Ferrari J, Darby AC, Daniell H, Godfray HCJ, Douglas A. Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance. Ecol. Entomol. 29: 60-65, 2004.

Ferrari J, Scarborough CL, Godfray HCJ. Genetic variation in the effect of a facultative symbiont on host-plant use by pea aphids. Oecologia 153: 323-329, 2007.

Gerardo NM, Altincicek B, Anselme C, Atamian H, Barribeau SM, de Vos M, *et al.* Immunity and other defenses in pea aphids, *Acyrthosiphon pisum*. Genome Biol. 11: R21, 2010.

Giordanengo P, Brunissen L, Rusterucci C, Vincent C, van Bel AJE, Dinant S, *et al*. Compatible plant-aphid interactions: How do aphids manipulate plant responses. CR Biol. 333: 516-523, 2010.

Gil R, Sabater-Munoz B, Latorre A, Silva FJ, Moya A. Extreme genome reduction in Buchnera spp.: Toward the minimal genome needed for symbiotic life. Proc. Natl. Acad. Sci. USA 99: 4454-4458, 2002.

Grenier AM, Duport G, Pagès S, Condemine G, Rahbé Y. The phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) is a pathogen of the pea aphid. Appl. Environ. Microbiol. 72: 1956-1965, 2006.

Guay JF, Boudreault S, Michaud D, Cloutier C. Impact of environmental stress on aphid clonal resistance to parasitoids: Role of *Hamiltonella defensa* bacterial symbiosis in association with a new facultative symbiont of the pea aphid. J. Insect Physiol. 55: 919-926, 2009.

Hansen AK, Moran NA. Aphid genome expression reveals host-symbiont cooperation in the production of amino-acids. Proc. Natl. Acad. Sci. USA 108: 2849-2854, 2011.

Helle W. Aphids, their biology, natural enemies and control, Vol. A, B, C. AK Minks and P Harrewijn Eds, Elsevier Science Publishers, 1987.

Henter HJ, Via S. The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. Evolution 49: 427-438, 1995.

Hufbauer RA, Via S. Evolution of an aphid-parasitoid interaction: variation in resistance to parasitism among aphid populations specialized on different plants. Evolution 53: 1435-1445, 1999.

Hullé M, Pannetier D, Simon JC, Vernon P, Frenot Y. Aphids of sub-Antarctic isles Crozet and Kerguelen: species diversity, host range and spatial distribution. Antarctic Sci. 15: 203-209, 2003.

Hurst GDD, Darby AC. The inherited microbiota of arthropods and their importance in understanding resistance and immunity. In: Rolff J, ST Reynolds ST (eds), Insect infection and immunity, Oxford University Press, 2009.

International Aphid Genomics Consortium. Genome sequence of the pea aphid *Acyrthosiphon pisum.* PLoS Biol,8(2): e1000313, 2010.

Laughton AM, Garcia JR, Altincicek B, Strand MR, Gerardo NM. Characterisation of immune responses in the pea aphid, *Acyrthosiphon pisum*. J. Insect Physiol. 57: 830-839, 2011.

Le Ralec A, Anselme C, Outreman Y, Poirié M, van Baaren J, Le Lann C, *et al*. Evolutionary ecology of the interactions between aphids and their parasitoids. CR Biol. 333: 554-565, 2010.

Li S, Falabella P, Giannantonio S, Fanti P, Battaglia D, Digilio MC, Völkl W, *et al*. Pea aphid clonal resistance to the endophagous parasitoid *Aphidius ervi*. J. Insect Physiol. 48: 971-980, 2002.

Login FH, Balmand S, Vallier A, Vincent-Monégat C, Vigneron A, Weiss-Gayet M, *et al.* Antimicrobial peptides keep insect endosymbionts under control. Science 334: 362-365, 2011.

Milner RJ. Distribution in time and space of resistance to the pathogenic fungus *Erynia neoaphidis* in the pea aphid *Acyrthosiphon pisum.* Entomol. Exp. Appl. 37: 235-240, 1985.

Montllor CB, Maxmen A, Purcell AH. Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. Ecol. Entomol. 27: 189-195, 2002.

Moran N, Baumann P. Phylogenetics of cytoplasmically inherited microorganisms of arthropods. Trends Ecol. Evol. 9: 15-20, 1994.

Moran N, Dunbar HE. Sexual acquisition of beneficial symbionts in aphids. Proc. Natl. Acad. Sci. USA 103 : 12803-12806, 2006.

Oliver KM, Russell JA, Moran NA, Hunter MS. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proc. Natl. Acad. Sci. USA 100: 1803-1807, 2003.

Oliver KM, Moran NA, Hunter MS. Variation in resistance to parasitism in aphids is due to symbionts not host genotype. Proc. Natl. Acad. Sci. USA 102: 12795-12800, 2005.

Oliver KM, Moran NA, Hunter MS. Costs and benefits of a superinfection of facultative symbionts in aphids. Proc. R. Soc. B 273: 1273-1280, 2006.

Oliver KM, Degnan PH, Hunter MS, Moran NA. Bacteriophages encode factors required for protection in a symbiotic mutualism. Science 325: 992-994, 2009.

Oliver KM, Degnan PH, Burke GR, Moran NA. Facultative symbionts of aphids and the horizontal transfer of ecologically important traits. Ann. Rev. Entomol. 55: 247-266, 2010.

Peccoud J, Simon JC, von Dohlen C, Cœur d'acier A, Plantegenest M, Vanlerberghe-Masuti F, *et al*. Evolutionary history of aphid-plant associations and their role in aphid diversification. CR Biol. 333: 474-487, 2010.

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, *et al*. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464: 59-65, 2010.

Sadd BM, Schmidt-Hempel P. Ecological and evolutionary implications of specific immune responses. In: Rolff J, ST Reynolds ST (eds), Insect infection and immunity, Oxford University Press, 2009.

Scarborough CL, Ferrari J, Godfray HC. Aphid protected from pathogen by endosymbiont. Science 310: 1781, 2005.

Schulenburg H, Kurtz J, Moret YT, Siva-Jothy M. Introduction. Ecological immunology. Philos. Trans. R Soc. Lond. B Biol. Sci. 364: 3-14, 2009.

Simon JC, Boutin S, Tsuchida T, Koga R, Le Gallic JF, Frantz A, *et al*. Facultative symbiont infections affect aphid reproduction. PLoS One, 6(7): e21831, 2011.

Slack E, Hapfelmeier S, Stecher B, Velykoredko Y, Stoel M, Lawson MA, *et al.* Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. Science 325: 617-620, 2009.

Schulenburg H, Kurtz J, Moret Y, Siva-Jothy MT. Introduction: ecological immunology. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364: 3-14, 2009.

Tagu D, Dugravot S, Outreman Y, Rispe C, Simon JC, Colella S. The anatomy of an aphid genome: from sequence to biology. CR Biol. 333: 464-473, 2010.

Toft C, Andersson SG. Evolutionary microbial genomics: insights into bacterial host adaptation. Nat. Rev. Genet. 11: 465-475, 2010.

Tsuchida T, Koga R, Fukatsu F. Host plant specialization governed by facultative symbiont. Science 303: 1989, 2004.

Tsuchida T, Koga R, Horikawa M, Tsunoda T, Maoka T. Matsumoto S, *et al*. Symbiotic bacterium modifies aphid body color. Science 330: 1102-1104, 2010.

von Burg S, Ferrari J, Müller CB, Vorburger C. Genetic variation and covariation of susceptibility to parasitoids in the aphid *Myzus persicae*: no evidence for trade-offs. Proc. Biol. Sci. 275: 1089-1094, 2008.

Vorburger C, Gouskov A, von Burg S. Genetic covariation between effectiveness and cost of defence in aphids. Biol. Lett. 4: 674-676, 2008.

Vorburger C, Sandrock C, Gouskov A, Castañeda LE, Ferrari J. Genotypic variation and the role of defensive endosymbionts in an all-parthenogenetic host-parasitoid interaction. Evolution 63:1439-1450, 2009.

Vorburger C, Gehrer L, Rodriguez P. A strain of the bacterial symbiont *Regiella insecticola* protects aphids against parasitoids. Biol. Lett. 6: 109-111, 2010.

Vorburger C, Gouskov A. Only helpful when required: a longevity cost of harboring defensive symbionts. J. Evol. Biol. 24: 1611-1617, 2011.

Wilson ACC, Ashton PD, Calevro F, Charles H, Colella S, Febvay G, *et al*. Genomic insight into the amino acid relations of the pea aphid, *Acyrthosiphon pisum*, with its symbiotic bacterium *Buchnera aphidicola*. Insect Mol. Biol. 19: 249-258, 2010.

Weiss BL, Wang J, Aksoy S. Tsetse immune system maturation requires the presence of obligate symbionts in larvae. PLoS Biol, 9(5): e1000619, 2011.

Xie J, Vilchez I, Mateos M. Spiroplasma bacteria enhance survival of *Drosophila hydei* attacked by the parasitic wasp *Leptopilina heterotoma*. PLoS One, 5(8): e12149, 2010.