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VISIONS AND PERSPECTIVES

Evolving aphids: one genome-one organism insects or holobionts?

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Abstract

Aphids have obligate mutualistic relationships with microorganisms that provide them with essential substances lacking in their diet, together with symbionts conferring them conditional adaptive advantages related, for instance, to the thermal tolerance and to the resistance to parasitoid wasps. The presence/absence of a secondary symbiont may have a relevant phenotypic effect so that aphid microbial symbionts constitute a sort of second genome with its own genetic inheritance. On the whole, genes important for aphid survival and reproduction are not uniquely present in the aphid nuclear and mitochondrial genomes, but also in the chromosomes of each symbiont. As a consequence, aphids should be viewed as holobionts with an extended genome (the hologenome) including the host and its symbiotic microbiome. In this connection, the true unit of selection in evolution must be considered the aphid holobiont, in place of the single host as individual separated from its symbionts.

Key Words: aphids; bacterial symbiont; symbiosis; hologenome; holobiont

Introduction

Aphids (Hemiptera: Aphididae) are ancient insects, whose fossils go back to the Triassic, about 220-210 myr ago (Grimaldi and Engel, 2005), that have conquered most of the biomes, including the arctic and subarctic regions and infest a huge range of plants (Loxdale, 2008a).

Aphids reproduce primarily by apomictic parthenogenesis, a form of reproduction whereby adult females give birth to a female progeny without any male fertilization (Soumalainen *et al.*, 1987; Loxdale, 2008a). Several authors frequently suggested that no genetic recombination occurs in the parthenogenetic generations (Soumalainen *et al.*, 1987; Spence and Blackman, 1998), so that it has been assumed that the aphid offspring represents a genetically identical clone of aphids (Dixon, 1989).

In 1977, Dan Janzen argued that clonal lineages of aphids could be considered as "evolutionary individuals" with the ability to exploit resources over a wide geographical area. These multiple individuals thereby have a competitive edge over single organisms that lack the capacity to propagate parthenogenetically (apomictically). Aphids can reproduce quickly and it has been calculated that under ideal conditions (absence of predators, parasites, pathogens and benign climatic conditions, especially including optimal temperatures of 20 -25 °C), a single asexual female could in theory produce 7.6×10^{28} offspring in a single growing season (with a generation given as 7 days, 50 offspring per female and 18 generations a year) (Harrington, 1994). However, whether they can maintain a long-term genetic fidelity, if at all, and how long so-called clones persist unchanged either in the laboratory or in the field is still a contentious issue (Loxdale, 2008a, b, 2009).

Actually, aphid lineages within a same species have been found to differ for colour (Loxdale, 2008b), size (Jenkins, 1991), intrinsic rate of increase (Jenkins, 1991), ovariole number (Dixon, 1989), reproductive modes (Loxdale, 2008b), ability to transfer pathogenic plant viruses (Terradot *et al.*, 1999) and susceptibility/resistance to predators, parasites, pathogens and pesticides (Losey *et al.*, 1997; Devonshire *et al.*, 1999; Loxdale, 2008b). These findings strongly suggest that clonality in aphids has been overestimated prompting an evaluation of the true nature and reality of the concept of clone (Loxdale, 2008a, b).

In the last years, a growing amount of molecular evidences suggested that aphid asexual lineages are not true clones, since they can rapidly

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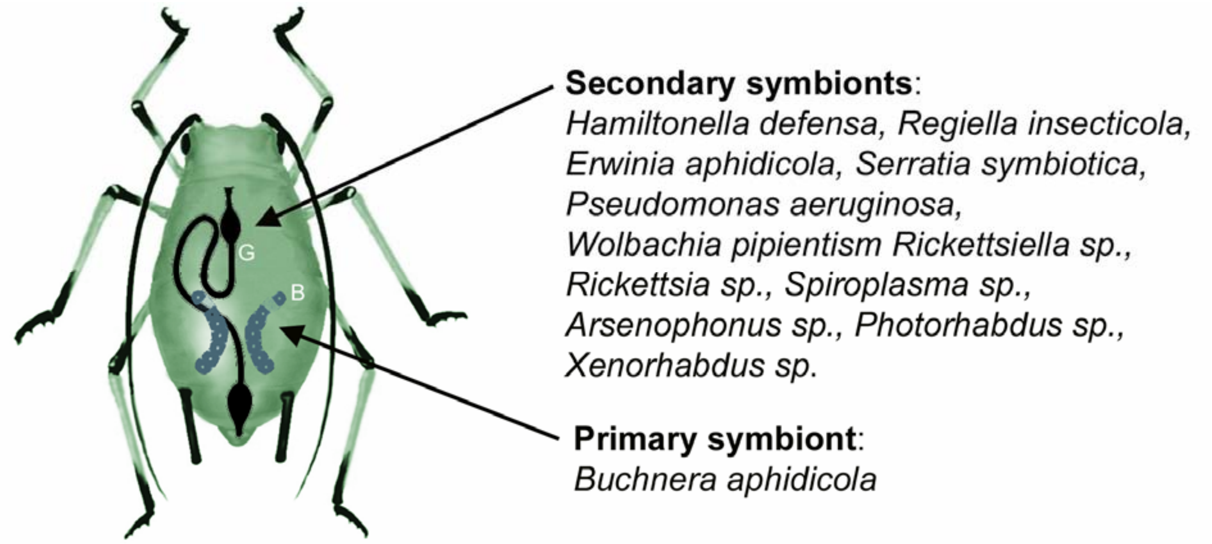

Fig. 1 Different microorganisms can be harboured in diverse aphid organs. The primary symbiont *B. aphidicola* is generally hosted in the bacteriocytes (B), specialized fat body cells located near the aphid gut (G). A highly diversified bacterial community has been identified in the aphid gut since differences in the microbiome have been observed not only comparing diverse species, but also making a comparison of different populations of the same species, but feeding on different plants.

mutate and this variation is selectable and may affect some phenotypic traits, such as the host choice (Loxdale, 2008a, b; Martens *et al*., 2009), so that the real nature of clone in aphids is not simply semantics. Indeed, the presence of genetic differences among clones could be very important for aphid evolution since several studies showed that cryptic sympatric speciation occurred in a wide range of aphid species (Loxdale, 2008a, b), including evidences of rapid chromosomal changes affecting speciation events in the aphids *Rhopalosiphum maidis* and *Myzus persicae* (Blackman, 1987; Brown and Blackman, 1988; Monti *et al*., 2012).

Starting from the hypothesis of the clonal reproduction of aphids, some authors referred to aphids as a single genome species suggesting not only that each aphid presents a clear correspondence one genome-one organism, but also accepting the idea that the aphid phenotype relies on the nuclear genome only (as recently revised in Loxdale, 2008a, b).

In view of their impact in agriculture (in particular for virus transmission), aphids need to be controlled by pesticides and/or using biological control agents. However, in the absence of a thorough understating of the genetics of aphid populations/clones, the identification of transmissible and adaptative variations could make biological and chemical controls not really effective (Loxdale, 2008b).

Same nuclear genome, different microbiome?

Aphids have close associations with various lineages of microorganisms and most of them may harbour, for instance, the obligate mutualist (usually called primary symbiont) *Buchnera aphidicola* (Russell and Moran, 2006). In addition to *B. aphidicola*, other maternally transmitted intracellular bacteria, such as *Rickettsia sp.* (α-Proteobacteria), *Spiroplasma sp.* (Mollicutes) and various γ-proteobacterial microbes (including *Hamiltonella defensa, Regiella insecticola, Serratia symbiotica* and *Arsenophonus sp.*), are harboured in aphids constituting their microbiome (Chen *et al*., 1996; Fukatsu *et al*., 2000, 2001; Gomez-Valero *et al.,* 2004) (Fig. 1).

Aphid secondary symbionts are often shared between divergent lineages and they seem to undergo both vertical and horizontal transfer among matrilines within and between species (Russell and Moran, 2006). This transmission is due to the ability of the secondary symbionts to overcome host immune responses and invade various types of host cells, including germ cells (Russell and Moran, 2006).

Several data suggested that symbiotic bacteria are involved in different traits of the aphid biology, including resistance to parasitoid wasps (Oliver *et al*., 2003), tolerance to heat stress (Montllor *et al.*, 2002) and changes in the host plant range (Tsuchida *et al*., 2004). Moreover, as also showed in other insects, symbionts may modulate other complex interactions protecting the host from the invasion by pathogenic microorganisms (a process known as “colonization resistance”) and modulating the aphid immune system (Russell and Moran, 2006; Poirié and Coustau, 2011). Microbiome seems therefore to act in aphids (and also in taxa other than insects) as a sort of ecological immunity or extended immune system being able of affecting the efficiency of the host immune system and limiting the accumulation of pathobionts. The role of the aphid microbiome could be particularly relevant since, as recently reviewed, the immune deficiency (IMD) signalling pathway was apparently non functional in aphids (Poirié and Coustau, 2011). Moreover, no genes coding for peptidoglycan recognition proteins (PGRPs) and several well-conserved antimicrobial peptides, such as defensins and cecropins, have been predicted in the pea aphid *Acyrthosiphon pisum* genome (Gerardo *et al*., 2010; Poirié and Coustau, 2011), making the microbiota-based immunity essential to protect aphids against natural enemies (Poirié and Coustau, 2011).

Different roles have been suggested for symbionts other than the synthesis of amino acids (Douglas *et al*., 1998). *Buchnera* might, for instance, play a key role in aphid thermal tolerance. Thermal tolerance of the primary endosymbiont *Buchnera* is attributed to genes coding for heat shock proteins, which deter degradation of protein secondary structure (Shigenobu *et al*., 2000). Interestingly, in the presence of low density of primary symbionts, secondary symbionts (such as *H. defensa*, *S. symbiotica* and *R. insecticola*) could be more numerous affecting the aphid thermal tolerance (Oliver *et al*., 2010). For instance, *S. 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 % in hot places (Oliver *et al*., 2010).

Secondary symbionts may change aphid colour, as showed for *Rickettsiella* that induces a body colour change from red to green affecting prey-predator interactions, since ladybird beetles preferentially consume red aphids, whereas parasitoids are more attracted by green ones (Tsuchida *et al*., 2010). Secondary symbiont can also affect the adaptation to the host plant as assessed for infections by *R. insecticola* that could improve aphids' fitness specifically on *Trifolium* plants (Tsuchida *et al*., 2004). Lastly, resistance to parasitoids is associated with secondary symbionts, as showed by several studies where the symbiotic associations have been experimentally manipulated either by suppressing symbionts using antibiotics treatment or by introducing a new symbiont through bacterial microinjection. According to literature data, *Aphidius ervi* parasitism success on *A. pisum* is lower in aphid lines harbouring *H. defensa* and *S. symbiotica* respectively (Oliver *et al*., 2003, 2005, 2006; Ferrari *et al*., 2004; Vorburger *et al*., 2009; Poirié and Coustau, 2011).

On the whole, secondary symbionts play different roles, but are they stable within an aphid species? Can they vary among populations? According to Sandström *et al*. (2001) the associations with secondary symbionts are quite variable, suggesting a rather labile relationship between aphid species and their secondary symbionts. Therefore, in contrast to the stable association between aphids and their primary symbionts (Moran *et al*., 1993; Clark *et al*., 2000), secondary symbionts can be lost due to infidelity of the vertical transmission or gained by horizontal transmissions. For instance, an *A. pisum* laboratory clone that lost two secondary symbiont types has been described in literature (Sandström *et al*., 2001), together with the identification in the field of aphid populations hosting multiple or single secondary symbionts (Sandström *et al*., 2001). Moreover, a differential resistance to braconid parasitoids has been described in populations of *A. pisum* (Hufbauer and Via, 1999; Ferrari *et al.,* 2001), *M. persicae* (von Burg *et al*., 2008) and *Aphis fabae* (Vorburger *et al*., 2009), suggesting that a differential composition of the microbiome strongly affect the survival and reproduction of aphids.

If we therefore accept that a large community of bacteria may invade aphids and that they are transmitted both vertically and horizontally within and among aphid lineages and that different populations may have different microbiomes, can we surmise without any doubt that each aphid within a clone harbours the same microbiome? In view of the horizontal transfer of symbionts and their beneficial effects on the reproductive success of parthenogenetic females, a single gain of a secondary symbiont can have beneficial effects on the aphid carriers influencing their fitness.

At this regards, Gehrer and Vorburger (2013) demonstrated a previously undescribed route of horizontal transmission consisting in the parasitoid-mediated transfer of endosymbionts among aphid clones by sequentially stabbing infected and uninfected aphids. The wasp’s ovipositor appears to act as a ‘dirty needle’ that can inoculate previously uninfected aphids. If the recipient aphid resists the parasitoid and survives the attack, this can result in a new, heritable infection. Considering that many aphid parasitoids use multiple hosts, it is likely that they can transfer symbionts not just within, but also between, aphid species (Gehrer and Vorburger, 2013).

Due to their relevant roles in aphid biology, the presence/absence of a secondary symbiont may have an important phenotypic effect so that aphid microbial symbionts form a true second genome with its own genetic inheritance (Moran, 2007; Gilbert, 2011). Furthermore, symbiotic bacteria provide a selectable allelic variation (thermotolerance, colour and parasitoid resistance) that enables aphids to persist under different environmental conditions so that mutations occurring in the symbiont genomes may affect aphid fitness and evolution (Dunbar *et al*., 2007; Tsuchida *et al*., 2010).

Aphids as holobionts

More than a hundred years of biological research demonstrated the importance of microorganisms in the health and disease of higher organisms, including humans (Ottaviani *et al*., 2011). As a result of the recent development of culture-free molecular techniques, it is now accepted that in many cases the number of symbiotic microorganisms and their combined genetic information far exceed that of their hosts so that for each gene in our genome, we host about

100 genes belonging to human bacterial symbionts (The Human Microbiome Project Consortium, 2012).

In view of this new view of the symbiotic interactions, Zilber-Rosenberg and Rosenberg suggested the hologenome theory of evolution defining hologenome as the sum of the genetic information of the host and its microbiome (Rosenberg *et al*., 2007; Zilber-Rosenberg and Rosenberg, 2008).

As a consequence of the hologenome theory of evolution, each organism should be viewed as an holobiont including the host and its symbiotic microbiome and the holobiont is the true unit of selection in evolution in place of the single host as individual separated from its symbionts (Rosenberg *et al*., 2007; Zilber-Rosenberg and Rosenberg, 2008). At this regards, it has to be underlined that relatively rapid variation in the diverse microbial symbionts can have an important role in the adaptation and evolution of holobionts identifying them as dynamic entities in which a vast amount of the genetic information and variability is contributed by microorganisms. In view of this assumption, the evolution of holobionts can occur by changes in the host genome and/or in any of the hosted microbial genome, and relies on the cooperation between the genomes within the holobiont, as much as on competition with other holobionts.

Similarly, genetic variation can arise from changes in either the host or the symbiont genomes. Variation in host genomes occurs during sexual reproduction, chromosome rearrangements and ultimately by mutations, but the same processes occur in microbial symbionts with the noteworthy difference that in haploid bacteria recombination occurs also by conjugation, transduction and DNA transformation among different species (Rosenberg *et al*., 2007; Zilber-Rosenberg and Rosenberg, 2008).

Concluding remarks

Contrarily to rare recombinations and mutations of the host genome, changes in the genetic information related to symbionts can occur quickly by microbial amplification, acquisition of novel bacterial strains and horizontal gene transfer between different species (including gene transfer from the symbiont to the host genome). In particular, the microbial amplification is the most rapid and easy mechanism to achieve variations in holobionts, since it involves changes in the relative numbers of the diverse types of associated microorganisms that can occur as a result of changing temperatures, nutrient availability, exposure to xenobiotics or other environmental factors (Moran, 2007; Rosenberg *et al*., 2007; Zilber-Rosenberg and Rosenberg, 2008; Gilbert, 2011). The main advantage for hosts is therefore that they can survive, multiply and gain the time necessary for their genome to evolve using the genetic information available in their symbionts (Moran, 2007; Gilbert *et al*., 2012).

As a whole, although Dan Janzen's (1977) original concept was fascinating and not implausible thirty years ago, the use of the one-genome/one-organism paradigm of classical genetics has been eclipsed by recent studies on symbiosis suggesting a revision of our approach to the aphid biology and evolution. Aphid clones cannot be considered as evolutionary individuals in any sense of the term neither the idea of aphids as a single genome species should be further considered in the light of the existence of the aphid hologenome. On the whole, also taking into account the peculiar structure of their immune system, aphids should be regarded as highly plastic organisms (probably more than other insects), whose evolution has been shaped by their symbionts.

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