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EDITORIAL

Is there a transgenerational inheritance of host resistance against pathogens? Lessons from the *Galleria mellonella-Bacillus thuringiensis* interaction model

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Introduction

Over the past 20 years, the explosion of research on epigenetics has provided a mechanistic basis for how the environment influences gene expression in living organisms, without acting on the nucleotide sequence of DNA. Epigenetic characters are carried by chemical modifications of chromatin (DNA methylation, post-translational modifications of histones) and/or by RNAs. Their role is fundamental in the determinism of cellular identity during embryonic development. They also shape the response of somatic cells to endogenous stimuli (e.g. hormones, growth factors, metabolites, cytokines) or exogenous stimuli (e.g. nutrients, toxic chemicals, microbial molecules, stress).[1] Some changes in chromatin are inheritable during cell divisions, allowing a stable transcriptional reprogramming of somatic cells over time. But can epigenetic variation be transmitted from a whole organism to the descendants? In other words, can epigenetic variation reach the germinal cells and cross the barrier of sexual reproduction, thus perpetuating new phenotypes induced by the environment, in absence of any genetic mutation? This "Lamarckian" question is the source of many interests and debates. While transgenerational epigenetic heritability is well established in plants,[2] its existence in animals still raises many questions.[3,4] Hence, it is of importance to generate experimental models enabling to follow complex phenotypes, together with genetic and epigenetic modifications, for many generations.

Some insect species can be used as such models, as they offer several advantages over mammalian models, including short generation times, ethical acceptability, and a potential for studying complex parameters (e.g. longevity, fertility, gender ratio, responses to environmental stresses).[5] Insect models are also powerful for studying microbial infections and host resistance against microbes. It should be emphasized that pathogens are capable of acting on the epigenetic machinery of their host, and conversely, epigenetic regulators have been identified as actors of the host responses to infections.[6,7] There is also an epigenetic component in the mechanistic basis of inter-individual differences in immune responses to pathogens.[8] It is therefore tempting to speculate that epigenetic mechanisms could play a role in the transmission of protective defenses against pathogens from parents to offspring. In this issue of *Virulence*,[9] Mukherjee *et al.* investigates this fascinating hypothesis by using the larva of the greater wax moth *Galleria mellonella* as a model host to study the transmission of the resistance against the entomopathogen *Bacillus thuringiensis*.

Repeated infections of Galleria mellonella with Bacillus thuringiensis: A *laboratory evolution model to study acquired resistance against pathogens.*

B. thuringiensis (Bt) is the most important bio-insecticide world-wide.[10] This gram-positive spore-forming bacterium produces Cry toxins that display powerful and specific insecticidal activity toward the larvae when ingested. Thousands of *B. thuringiensis* strains and hundreds of Cry toxin-encoding genes have been identified (http://www.btnomenclature.info/), but only a few are commercially used. The mode of action of Cry toxins involves a solubilization step of the toxin crystals in the gut, followed by an enzymatic activation of the protoxin. Then, the active form binds to membrane proteins of intestinal epithelial cells (e.g. alkaline-phosphatase, amino–peptidase, lipoproteins), resulting in cell intoxication and osmotic lysis.[11,12] Some Cry toxins can kill the insect on their own, as shown in transgenic Bt–crops expressing *cry* genes.[13]The lethal effect depends on the

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ingested amount of toxin and the size of the larva. However, the presence of Bt bacteria in the insect gut is generally required to potentiate the toxin effect. Indeed, Bt bacteria encode several other virulence factors, such as hemolysins, phospholipases and metalloproteases, which are produced in the vegetative bacteria and are important for infection.[14,15]Among Bt target species, *Galleria mellonella* (greater wax moth or honeycomb moth) is particularly useful to study the relative importance of Bt bacteria, since in this insect the presence of Bt is required for full virulence.[16] In addition, *G. mellonella* larvae can be produced inexpensively in large numbers and are simple to use in laboratory conditions.[17] Their short life cycle makes them ideal for large-scale studies during many generations and their innate immune response exhibits similarities with their vertebrate counterpart.[18,19]

In all kinds of biocidal activities, it is well known that the organism under pressure will do its best to tolerate the presence of the biocidal compound. Insects are particularly efficient at developing mechanisms of resistance against pesticides, bacterial pathogens or toxins. In the particular case of resistance to Cry toxins, the reduced susceptibility of the host is often due to alterations of components of the toxin lytic pathway, for example via mutations in the binding sites of the toxin receptor or via the absence of receptor on the surface of intestinal cells.[11] Deregulating genes involved in host defense mechanisms, such as genes encoding antimicrobial peptides, is also a means of becoming resistant to mixtures of Bt Spore-Cry.

In a pioneer study, Dubovskiy *et al.*[20] developed an evolutionary model focused on the resistance of *G. mellonella* against Bt spore-Cry mixtures. After a long-term selection process for 20 generations, they obtained a resistant *Galleria* line, which was about 9-fold more resistant to the pathogen than the non-selected control. Study of the expression of a set of selected genes, based on *G. mellonella* available transcriptome contigs and sequenced genes,[21] as well as the analysis of the gut microbiota, highlighted the immuno-physiologic adaptation of this Bt-resistant *G. mellonella* line, both in the midgut and fat body cells. In particular, the resistant colony exhibited higher expression levels of genes coding for antimicrobial peptides and stress response proteins. The colony also displayed a better fitness (i.e. a higher fecundity) and a decrease in the diversity of the gut microbiota.[20]

Change in epigenetic patterns in a Galleria resistant line after 30 generations of selection.

In the follow-up study published in this issue[9] Mukherjee *et al.* extended the selection process up to 30 generations, leading to a selected line exhibiting about 11-fold enhanced resistance against Bt spores-Cry mixtures compared with the susceptible control. Using antibody-based approaches to quantify the global amount of methylated DNA and acetylated histones, they observed that the levels of 5-methylcytosines and acetylated histones were higher in the midgut and the fat body of resistant larvae than in susceptible larvae. These effects coincided with changes in expression levels of host genes encoding epigenetic modifiers, including DNA methyltransferases, histone deactelylases and histone acetyltransferases. The authors also observed differences in the expression of a set of miRNAs. This study thus shows that the shift in resistance is associated with altered epigenetic mechanisms. It lays the foundation for a role of epigenetic regulations in *Galleria* defense to Bt. However; there is still a long way to demonstrate that the acquisition of resistance during many generations is based on the transmission of epialleles. First, one cannot rule out that the observed changes in epigenetic marks are due to indirect effects resulting from modifications of the genotype. For example, mutations in genes encoding transcription factors or signaling molecules may impact the level of expression, as well as the efficiency and localization, of chromatin-remodeling complexes, leading to changes in the chromatin landscape. To demonstrate that altered chromatin states can be transmitted to *Galleria* progenies along many generations, a prerequisite is to sequence and compare the genome of the parental line and the progeny lines selected in presence or absence of the pathogen challenge. Unfortunately, the sequence of the *G. mellonella* genome is not yet available. The genome sequence is also required to extensively scan the epigenome with highly precise techniques, such as Chromatin Immunoprecipition sequencing and Bisulfite sequencing, to identify potential epimutations. Another important issue is to understand how the resistance trait is transmitted to the germ line and passed to the fertilized egg.

A progress in the demonstration of the existence of transgenerational epigenetic mechanisms in insects has recently been made in the flagship model *Drosophila melanogaster*. The Cavalli laboratory demonstrated that the spatial architecture of the genome controlled by Polycomb epigenetic factors can affect the pigmentation of the eyes of progenies in absence of genotype alterations.[22] In the framework of research on infectious diseases, it would be very interesting to bring epigenetic studies in *G. mellonella* to the level of those conducted in *Drosophila*. It is important to underline that *Galleria* have undeniable advantages over *Drosophila* to model infection by mammalian pathogens, since *Galleria* larvae tolerate

temperatures of and above 37°C and display a larger size than *Drosophila*. The larval stage of *G. mellonella* is used to address mechanisms involved in the pathogenesis of an increasing number of mammalian pathogenic bacteria and fungi[23,19] and to identify new antimicrobial components. It has for instance been used to evaluate phage therapy against *Clostridium difficile*[24] and septicemia triggered by *Listeria monocytogenes*.[25] It has enabled the analysis of chromosomal virulence factors in the *Bacillus cereus* group bacteria, to which Bt and the anthrax agent *Bacillus anthracis* belong.[26,15,17] Moreover, *G. mellonella* is often used as a trap for various insect pathogens (virus, fungi, nematodes, bacteria).

The understanding of the tolerance mechanisms of *Galleria* to Bt-Cry biocides has improved significantly thanks to the resistance evolution model in *Galleria*[20,9] There is now a need to explore in detail the relative importance on the selected resistance phenotype of Cry toxins and the other virulence factors expressed by Bt during infection.[15] Another important issue raised by this work is whether similar phenomena occur in insect pests, which are of high economical importance, such as *Plutella xylostella*.[27] Indeed a recent work highlights that the deregulation of the *trans*-regulatory effector MAP4K4 of *P. xylostella* influences the expression of Alkaline phosphatase and ABC binding cassette transporter, both known as receptors for Cry toxins.[28] Therefore, one may wonder if the dMAP kinase is due to epigenetic modifications, which could be transferred to progeny. The notion of transgenerational epigenetic inheritance can also be applied to other pathologies, triggered by a combination of genetic and environmental factors, such as metabolic diseases and neurologic diseases. Research on these complex diseases should benefit from various insect models, including *Drosophila* and *Galleria*.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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