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A Critical Study of Co-evolution of parasitic fungi and insect hosts

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Abstract :

Parasitic fungi and their insect hosts provide an intriguing model system for dissecting the complex co-evolutionary processes, which result in Red Queen dynamics. To explore the genetic basis behind host–parasite coevolution we chose two parasitic fungi (Beauveria bassiana and Metarhizium anisopliae, representing the most important entomopathogenic fungi used in the biological control of pest or vector insects) and two established insect model hosts (the greater wax moth Galleria mellonella and the red flour beetle Tribolium castaneum) for which sequenced genomes or comprehensive transcriptomes are available. Focusing on these model organisms, we review the knowledge about the interactions between fungal molecules operating as virulence factors and insect host-derived defense molecules mediating antifungal immunity. Particularly the study of the intimate interactions between fungal proteinases and corresponding host-derived proteinase inhibitors elucidated novel coevolutionary mechanisms such as functional shifts or diversification of involved effector molecules. Complementarily, we compared the outcome of coevolution experiments using the parasitic fungus B. bassiana and two different insect hosts which were initially either susceptible (Galleria mellonella) or resistant (Tribolium castaneum). Taking a snapshot of host–parasite coevolution, we show that parasitic fungi can overcome host barriers such as external antimicrobial secretions just as hosts can build new barriers, both within a relatively short time of coevolution.

Introduction

Host–parasite coevolution is a special form of coevolution involving reciprocal adaptive genetic changes in two antagonists, i.e. the particular host and parasite species, based on the selective pressures each partner confers on the other. The Red Queen hypothesis proposing continuous adaptation merely to maintain parity with other evolving species (Van

Valen, 1973) is clearly demonstrated by the arms race between hosts and parasites (for a description of different modes within the Red Queen, compare Brockhurst et al., 2014). The inspiration for this hypothesis is the following quote: "Now, here, you see, ittakes allthe running you cando,to keep inthe same place" (Carroll, 1871). However, the runners during coevolution do not keep pace − instead, one or the other may always be ahead. This involves adaptation and counter-adaptation, and it is most interesting to understand how the evolution of resistance in the host is matched by the evolution of virulence in the parasite, and vice versa. Insects and other invertebrates represent excellent model hosts because of their short generation times, allowing us to consider evolutionary strategies against a range of pathogens and parasites, including helminths, protozoa, bacteria and even viruses. For simplicity in the following we will refer to all these organisms as parasites.

One well-known study system for host–parasite coevolution, and in support of the Red Queen hypothesis, is the aquatic invertebrate Daphnia spp. and its parasites (reviewed in Ebert, 2008). In this same system Decaestecker et al. (2007) took advantage of the natural preservation of spatiotemporal interactions in different layers of lake sediment. This natural preservation is not possible in most systems, so researchers have turned to models that can be reared and tested in experimental settings. Experimental host–parasite coevolution is a powerful tool to investigate the evolution of host defense mechanisms and resistance as well as parasite virulence factors (e.g., Schulte et al., 2010; Masri et al., 2015; see Brockhurst and Koskella, 2013 for a review). Samples taken after different time points provide insight into evolutionary adaptations and counter-adaptations and such experiments have been carried out using model hosts such as Caenorhabditis elegans (Schulte et al., 2010; Masri et al., 2015), Daphnia spp. (Carius et al., 2001) and Tribolium castaneum (Wegner et al., 2008). Most of these experiments have considered bacterial parasites (but see Wegner et al., 2008), whereas fungi are neglected parasites of insects despite their importance (Madelin, 1966). Furthermore, as resistance against pesticides is spreading through populations of insect pests and vectors (Metcalf, 1989; Hemingway and Ranson, 2000), the search for alternative biological pest control agents is becoming more important. One such approach is indeed the application of entomopathogenic fungi(Ferron, 1978), which are natural parasites of insects (Nikoh and Fukatsu) that share several million years of coevolution with their hosts, making them ecologically important natural regulators of insect populations in almost all ecosystems (Ortiz-Urquiza et al., 2015).

We here review coevolution experiments using the entomopathogenic fungus Beauviera bassiana as parasite (Dubovskiy et al., 2013a,b; Rafaluk et al., unpublished data),

which has been established as a “model parasite” for the screening of genes involved in virulence (Valero-Jimenez et al., 2016). Coevolutionary outcome differed substantially depending on whether a susceptible host (the greater wax moth, Galleria mellonella) or a resistant host (the red flour beetle, Tribolium castaneum) was used. The susceptible host became resistant during the course of the experiment due to increased host resistance, whereas the resistant host became more susceptible because the parasite became more virulent. Both approaches yield important insight into the nature of adaptation and counter-adaptation and increase our understanding ofthe evolution of virulence and resistance.

Red Queen dynamics between fungal parasites and their insect hosts In order to explore the genetic basis of host–parasite coevolution we selected two parasitic fungi and two model host insects for which either completely sequenced genomes or comprehensive transcriptomic data are available. Beauviera bassiana and Metarhizium anisopliae are the most important parasitic fungi used as biological control agents against vector and pest insects. M. anisopliae was the first entomopathogenic fungus for which a whole sequenced genome became available (Gao et al., 2011; Staats et al., 2014). The genomic sequence of B. bassiana revealed similar adaptations to the parasitic life style as that of M. anisopliae (Xiao et al.,2012) Both fungi can kill a wide range of insects but occur in a variety of strains, which exhibit pronounced virulence against particular insect hosts. These fungi also share the ability to infect their insect hosts directly through the cuticle (Vilcinskas and Götz, 1999; Gillespie et al., 2000). For our comparative.

our comparative analysis of host–parasite coevolution between parasitic fungi and their host insects we selected the greater wax moth G. mellonella and the red flour beetle T. castaneum. The latter is a pest of stored products that occurs worldwide and has emerged as a model in developmental, molecular and evolutionary biology. It has therefore been selected as the first beetle for which a complete genome has been sequenced (Tribolium Genome Sequencing Consortium, 2008). Its immune gene repertoire has been analyzed by different methodological approaches (Zou et al., 2007; Altincicek et al., 2008). And it became a valuable modelhostfor testinghypotheses addressing interactions ofinsects with pathogens and transgenerational immune priming (Roth et al., 2010; Knorr et al., 2015 Milutinovic´ et al., 2016).

s a counterstrategy against the presence of inducible antifungal peptides in insects these parasitic fungi have independently evolved the ability to suppress innate immune responses in the infected host (Vilcinskas and Matha, 1997a,b; Vilcinskas et al., 1997a,b). Aside from the suppression of the AMP synthesis in the infected host, entomopathogenic

fungi produce virulenceassociated proteinases capable of both digesting host defense molecules including AMPs and suppressing its cellular immune responses (Griesch and Vilcinskas, 1998). The countermeasures of insects against fungal infection encompass the co-option of inducible peptidic inhibitors for antifungal immune responses to neutralize fungal proteases operating as virulence factors (Vilcinskas and Wedde, 1997). The simultaneous synthesis of AMPs along with peptidic inhibitors against fungal proteinases during immune responses results in their synergistic activity againstfungi. The intimate interaction between virulence-associated proteinases of parasitic fungi and immunity-related proteinase inhibitors of G. mellonella provides the possibility to study host–parasite coevolution directly at the frontline. We will address this topic in more detail in Section 3.

Another interesting fact is that B. bassiana and M. anisopliae are known to produce secondary metabolites, e.g. cyclic peptides

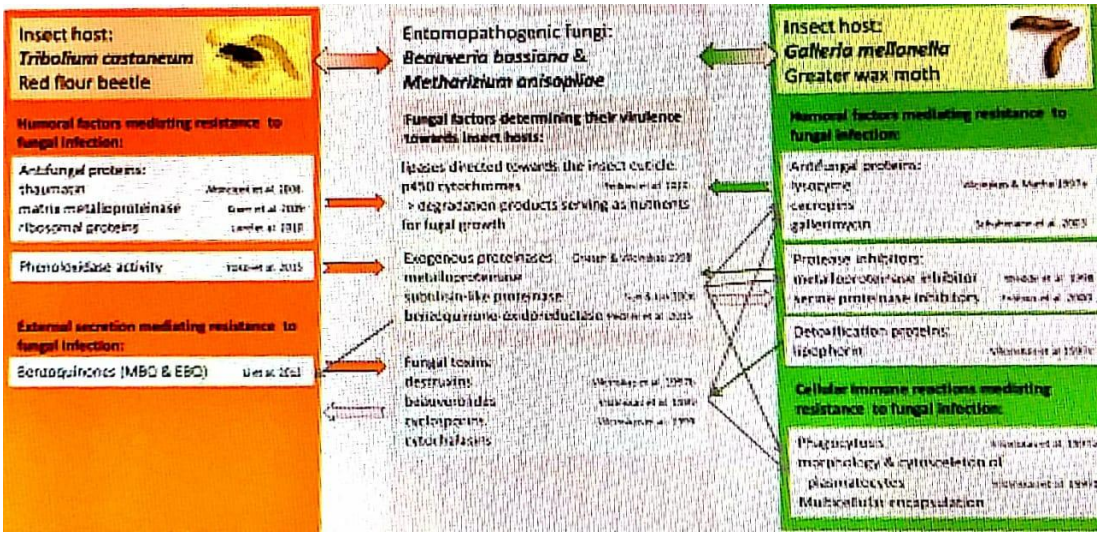

Fig. 1. Depicting the arms race of parasitic fungi with their insect hosts on the molecular level.As discussed in the text,the number of arrows represents the current snapshot of coevolution, with Tribolium castaneum currently being resistant and Galleria mellonella being susceptible to parasitic fungi. Abbreviations: MBQ, methyl-para-benzoequinone; EBQ, ethyl-para-benzoequinone. (Additional references cited in the figure: Sun and Liu, 2006, Lord et al., 2010, Pedrini et al., 2010).

such as beauverolides, cyclosporines or destruxins whichhave been implicated in contributing to the suppression of immune response in the infected host(Vilcinskas et al., 1999). For example, destruxins produced by M. anisopliae have been shown to induce apoptosis in immune competent hemocytes of G. mellonella (Vilcinskas et al., 1997b). Cyclosporin A, which is produced by B. bassiana and other entomopathogenic fungi, inhibits P-glycoprotein which mediates detoxification by removing a variety of toxic compounds from cells.

Inhibition of this membrane-associated ATP-dependent efflux pump in an infected insect hosts promotes its poisoning by toxic compounds taken up, e.g., with its plant diet (Podsiadlowski et al., 1998). Despite the high toxicity of cyclosporin A for dipterans, G. mellonella was found to be highly resistant even to high concentrations which were either injected or orally administered. The resistance of G. mellonella against this fungal toxin has been attributed to its lipophorins, which mediate binding and storage of the compound in the fat body in a physiologically inactive form (Vilcinskas et al., 1997c).

In contrast to the lepidopteran G. mellonella, the antifungal defense of the beetle T. casteaneum against fungi encompasses phenoloxidase activity (Yokoi et al., 2015), antifungal peptides (Altincicek et al., 2008) and secreted quinones (Li et al., 2013; Pedrini et al., 2015). The latter are produced by stink glands and mediate the sanitation of the microhabitat (Joop et al., 2014).

Experimental coevolution with an initially susceptible host

We have successfully established G. mellonella infected with the entomopathogenic fungi B. bassiana and M. anisopliae as a model system for investigating host–parasite coevolution, including the molecular level, focusing on the evolutionary dynamics between parasite-associated proteinases operating as virulence factors and host proteinase inhibitors involved in antifungal defense (Vilcinskas and Götz, 1999; Vilcinskas, 2010; Dubovskiy et al., 2013a,b). G. mellonella is susceptible to entomopathogenic fungi, and infection causes black spots to appear on the cuticle which represent the local activation of phenoloxidases to counteract fungal proteases resulting in the formation of melanin (Vilcinskas, 2010).

Experimental coevolution with an initially resistant host

We also established an experimental system for the analysis of host–parasite coevolution in T. castaneum and B. bassiana (Rafaluk et al., unpublished data), the former representing a resistant host in contrast to G. mellonella. The system was established to better understand by which means this host has evolved resistance to most parasitic fungi (Akbar et al., 2004; Khashaveh et al., 2011; Golshan et al., 2014). T. castaneum adults, in addition to the standardinsectimmune defenses, secrete antimicrobial substances that spread from the cuticle into the environment (Tschinkel, 1975; Unruh et al., 1998; Yezerski et al., 2007; Li et al., 2013). This provides communal protection, i.e. larvae in an environment previously conditioned by adult secretions are better protected against a natural parasite than larvae in an unconditioned environment (Joop et al., 2014). However, the adult secretion is toxic to the larvae (Omaye et al., 1981), so protection against pathogens comes at the cost of increased larval mortality in the presence of the secretion alone, depending on the environmental

concentration (Joop et al., 2014). Quinones represent a major component ofthe secretion (Villaverde et al., 2007; Li et al., 2013), and these redox-active molecules are knownto generate reactive oxygenspecies (ROS)thatinduce oxidative stress by oxidizing macromolecules such as lipids, proteins and nucleic acids, the latter associated with aging and carcinogenesis (Bolton et al., 2000; Li et al., 2013), by silencing candidate genes linked to quinone production, could show that this external defensive secretion provides an effective barrier against parasitic fungi, as beetles after RNAi were unable to inhibit fungal growth. Strategies used by parasitic fungito gain virulence against T

Conclusion :

Our comparative analysis of coevolution experiments with two distinct parasitic fungi and two model host insects enabled us to elucidate opposing dynamics that can result from different baseline situations. In the G. mellonella–B. bassiana system, the fungus was initially ahead but the insect host was able to close the gap, whereas in the T. castaneum–B. bassiana system, the beetle was initially ahead, but the fungus was able to draw level or even outpace the insect host. We can only speculate what we would have seen if these experiments had been run for a longer period, with regular sampling, but presumably the race would have continued, with host and parasite taking turns to lead. Taking a snapshot, i.e. stopping coevolution at a defined point, provides an opportunity to identify host barriers and parasite virulence factors at a given moment, and to compare these to the situation at the start of the experiment(also see Leal, 2015). Many factors at the baseline situation such as the population size or non-detected infections with other pathogens can influence the outcome of host–parasite coevolution (Papkou et al., 2016; Bose et al., 2016). Tolerance may also be of importance (Kutzer and Armitage, 2016). Understanding host defenses, parasite virulence factors and their evolution will help us to fight diseases, develop new drugs and my coin secticides, and avoid the emergence of resistant populations of parasites, pathogens and pests by, e.g., more carefully choosing pesticides and adjusting how we apply these. Even our basic results may have useful applications; e.g., selecting B. bassiana strains that tolerate oxidative stress could provide broader strategies for pest control, and these findings could also be transferred to other entomopathogenic fungi.

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