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NEWS

PERSPECTIVE

Unlocking the genetic basis of monarch butterflies' use of medicinal plants

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If there was any doubt of the primary role that plant secondary metabolites play in host–parasite co-evolution, the "From the Cover" paper by Tan et al. (2019) featured in this issue of *Molecular Ecology* will lay these doubts to rest. The group's previous work on monarch butterflies (*Danaus plexippus*) infected with the protozoan pathogen *Ophryocystis elektroscirrha* (OE) demonstrated higher survival and lower spore load on high cardenolide-producing milkweed (*Asclepias curassavica*) (Figure 1a) compared with low cardenolide-producing milkweed (*A. incarnata*) (de Roode, Pedersen, Hunter, & Altizer, 2008) (Figure 1b). The mechanism of this protective effect is not directly clear, but a leading hypothesis is that the cardenolides confer protection through toxicity to the parasite. However, the role of the caterpillar immune system in managing this parasite is largely unknown. Novel insights into the influence of toxic plant metabolites on caterpillar immunity are explored in Tan et al. (2019). Using transcriptomics to probe this model system, the authors found that herbivore immune genes were down-regulated and detoxification genes were up-regulated when larvae were reared on the milkweed species with high cardenolide concentrations (*A. curassavica*). Surprisingly, immune genes were not significantly up- or down-regulated in response to protozoan infection alone. This tantalizing result suggests that sequestered plant metabolites, not immunity, is reining in protozoan infections in these larvae, and promoting survival. As the authors point out, the strategy to invest in sequestration may come at a cost, which is to the detriment of the immune response (Smilanich, Dyer, Chambers, & Bowers, 2009). However, the cost becomes worth the investment when chemical sequestration takes on an antipathogen role. The novelty of the Tan et al. (2019) paper is that they show the investment in sequestration leading to a possible divestment in immunity.

KEYWORDS
coevolution, immunity, parasite, sequestration, transcriptomics

A comparison between the two milkweed species revealed a total of five immune genes that were down-regulated in larvae reared on the high-cardenolide species (Figure 1). The authors acknowledged that this was not a large number of immune genes that were down-regulated (four in gut tissue, one in the rest of the body). However, if the products of these immune genes are resource-expensive, then individuals with less investment in immunity can rely instead on sequestered cardenolides, which should be favoured by selection. From the data here, this selection would only have occurred when individuals were feeding on the high-cardenolide host plant because the down-regulation does not occur on the low-cardenolide host plant. This evidence also suggests that down-regulation is

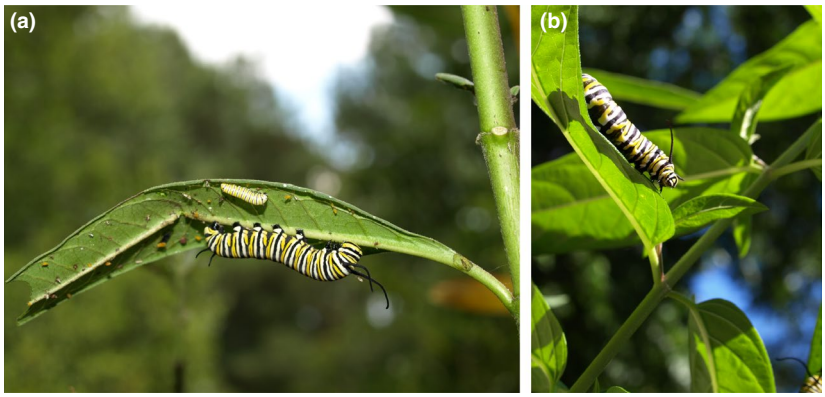

FIGURE 1 (a) Monarch caterpillars feeding on *Asclepias curassavica*, a high-cardenolide milkweed; Jacobus C. de Roode, Emory University. (b) Monarch caterpillars feeding on *Asclepias incarnata*, a low-cardenolide milkweed; Jacobus C. de Roode, Emory University

a plastic response that can be induced from feeding on particular host plants. One of the immune genes that was down-regulated on the high-cardenolide plant was a FREP-like receptor protein. The function of similar proteins in mosquito taxa is to defend against a protozoan parasite that attacks the mosquito host midgut much like the OE pathogen does in monarchs. The specificity of this down-regulated gene on the high-cardenolide host plant lends credence to the idea that cardenolides are functionally replacing immunity (Figure 2).

What has yet to be explicitly tested is whether cardenolide sequestration causes down-regulation of immunity directly, or if another cue associated with feeding on the high-cardenolide plant leads to a down-regulation of immunity. The simplest explanation would be that the amount of cardenolides or the unique composition of cardenolides act as the cue, given that these are the compounds that are putatively protecting the individuals against the protozoan parasite. What is curious about this is that down-regulation occurs regardless of infection status. Does this mean that the probability of parasite infection is so high it outweighs the potential cost of down-regulating immunity in a healthy individual? Or does it suggest that sequestration of high concentrations of cardenolides is energetically expensive, immune genes are down-regulated to offset the cost, and protection against parasites is a fortuitous consequence? Both of these are exciting possibilities with interesting implications for the evolution of sequestration (Petschenka & Agrawal, 2016) and self-medication (de Roode & Hunter, 2019).

Given the higher likelihood of survival from the protozoan infection on the high-cardenolide milkweed, it cannot be overlooked that the diet breadth of monarchs could be moulded by interactions with the OE pathogen and dietary chemistry. The frequency of infection by OE can average 70% in certain populations (Altizer, Oberhauser, & Brower, 2000), so using the right host plant matters. From the work by Tan et al. (2019), the evolutionary story may simply be that higher sequestration leads to greater protection from infection, and therefore using the high-cardenolide host plant is selected for. Individuals on the low-cardenolide host plant up-regulated ABC transporters (which have been linked to secondary metabolite sequestration) in an apparent attempt to increase the rate of sequestration to concentrate cardenolides. This is more evidence that the cardenolide chemistry is a major factor structuring the interaction with monarchs, their host plants and their natural enemies.

The use of transcriptomics to study this classic plant–herbivore–parasite model system opens the door to many additional questions previously inaccessible to the tools of molecular biology. From a purely descriptive standpoint, this study has confirmed the role of numerous monarch homologues of anticipated detoxification and sequestration genes. However, it is clear from the high number of differentially expressed "uncharacterized" genes that a great deal remains to be explored to understand detoxification mechanisms in lepidopterans, and sequestration in particular (Erb & Robert, 2016). In addition, the transcriptome data generated from Tan et al. (2019) could be mined to discover novel parasite genes associated with invasion of its host. In particular, how *O. elektroscirrha* stealthily achieves infection, apparently without triggering a robust immune response from the host, is worthy of further investigation. The work by Sternberg et al. (2012) revealed host plant-based tolerance of the OE parasite with cardenolide chemistry explaining a large proportion of the tolerance variation between host plant species. Nonetheless, the precise mechanism of this tolerance remains to be identified.

Avenues of future research pursuits in this area of study include dissection of the sequestration/parasite relationship. Studies are beginning to accumulate that show other sequestered plant metabolites are also beneficial for tolerating, reducing or eliminating parasite infections in other systems (del Campo, Halitschke, Short,

FIGURE 2 An adult monarch butterfly; Jacobus C. de Roode, Emory University

Lazzaro, & Kessler, 2013; Singer, Mace, & Bernays, 2009; Sternberg et al., 2012), but the mechanisms underlying this process remain a black box. Carefully designed lab experiments that make use of lepidopteran cell cultures (e.g., Sf9 cells) with cultured parasites and added plant metabolites could be a first step in isolating the antiparasitic effect of particular compounds. Clearly, the immune system adds another layer of complexity to the interaction. The lack of differential immune gene expression between the infected and uninfected individuals in Tan et al. (2019) suggests that immune variation at the transcript level might not be a reliable indicator of an immune response to certain pathogen infections. Although transcriptomics is an important tool, the authors note several situations in which it may overlook immune responses. For instance, localized immune responses may be undetectable when applied at a whole-body or large tissue scale (such as the midgut); immune responses may be muted in developing younger instars with immature immune systems; and single collection time points during infection aetiology may miss critical stages in the immune transcriptional response. Post-transcriptional measures (e.g., encapsulation, phenoloxidase activity, antimicrobial peptides) might therefore be more predictive parameters in this situation. Nonetheless, given the general hypotheses of how the immune system might respond to sequestered metabolites and parasites (see Tan et al., 2019), then the inclusion of immunity will certainly lead to better revelations of the study system.

AUTHOR CONTRIBUTION

AMS and ABN contributed equally to writing the manuscript.

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