Evolution of defences in large tropical plant genera: perspectives for exploring insect diversity in a tri-trophic context

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Graphical Abstract
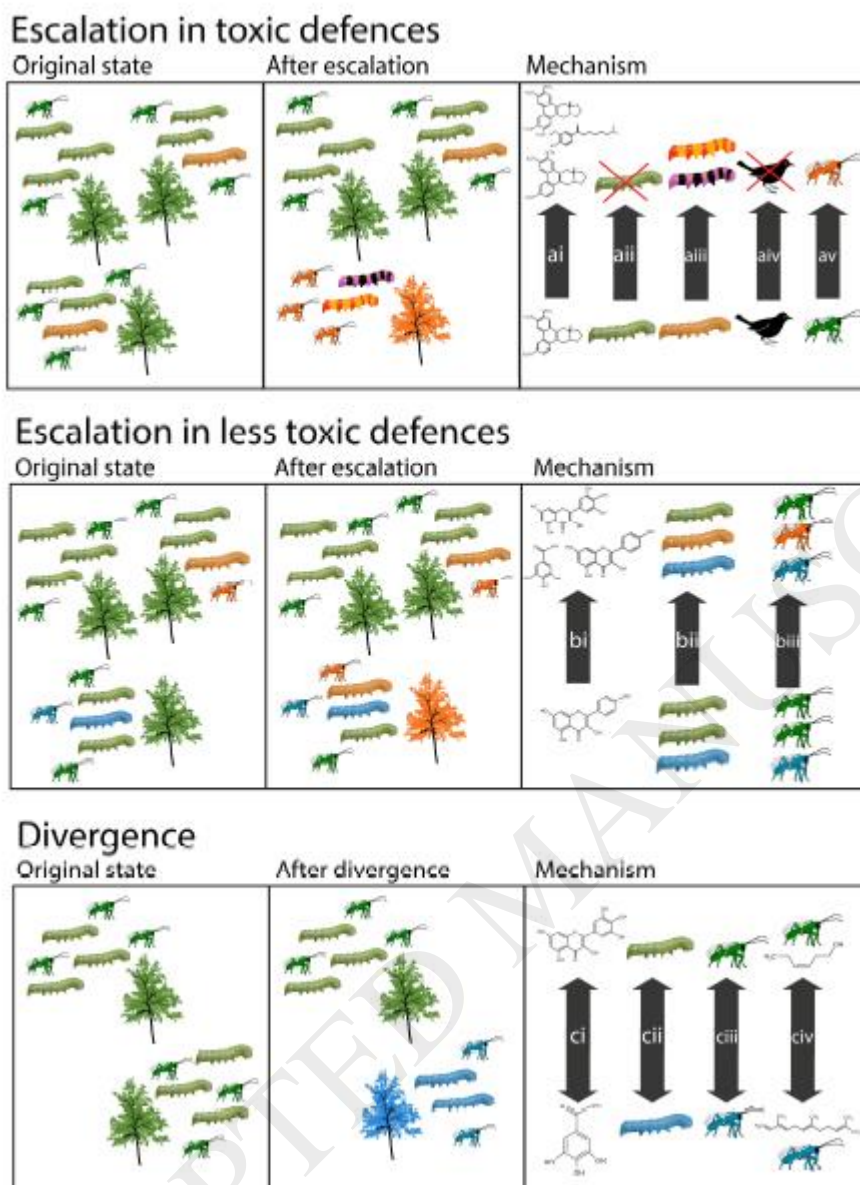
Proposed effects of evolutionary trends in host plant defences on insect diversity. Escalation in toxic defences (ai) frees hosts from herbivory by generalist herbivores (aii). But it can favor speciation of specialized, sequestering herbivores (aiii). The sequestration can lower attractivity of such herbivores to predators (aiv) or disrupts their physiological defences making them preferred by specific parasitoids (av). In contrast, escalation in less toxic defences (bi) probably does not lead to exclusion of herbivores from the community, but rather shifts relative proportion among species, increasing the diversity of herbivores associated with the respective host (bii). This may have a positive cascading effect on parasitoid diversity (biii). A divergence in direct defences (ci) lowers the amount of herbivores the hosts share and promotes herbivore community variation between the hosts (cii). This cascades to parasitoids, promoting variation in their communities as well (ciii). A divergence in HIPVs can further promote the effect (civ).

Highlights

- Large plant genera harbour a substantial share of the global insect diversity.
- This is because of their chemical diversity, promoted by divergence and escalation.
- This primarily affects the diversity of herbivores and cascades to parasitoids.
- Novel measures of chemical diversity may help to understand the mechanism.
- These need to be combined with detailed herbivore and parasitoids data.

Abstract

Divergence and escalation in defences promote chemical diversity in plants, and consequently the diversity of insect herbivores. This diversification cascades to insect parasitoids through direct effects on host herbivore susceptibility, changes in herbivore community composition, or disparity in plant volatiles. Large tropical plant genera represent an ideal model for studying these trends due to the high diversity of sympatric species and their insects. Novel measures of chemical structural similarity should be used to analyse evolutionary trends in both direct and indirect defences. Host chemical data need to be combined with detailed herbivore and parasitoid data. This will help to identify truly active compounds. Furthermore, resolved genomic phylogenies for plants and insects should be included to assign directionality in the processes.

Key words

coevolution, divergence, diversification, escalation, herbivory, parasitoids, sequestration

Introduction

Large tropical plant genera have long fascinated biologists [1,2]. This is because high abundance and diversity make genera such as *Psychotria* (ca 1,850 species), *Piper* (>1,000 species) or *Ficus* (ca 800 species) a key component of forest communities in both the Paleo- and Neotropics [2,3,4]. Such large tropical plant genera have been shown to harbour hundreds of insect herbivore species locally and thousands globally [3,5]. Exceptionally high herbivore diversity cascades to higher trophic levels as specialized herbivores sustain parasitoid and predator populations [6].

The diversity of insect communities associated with large tropical plant genera is linked to the chemical diversity of the hosts [e.g. 7*,8-10*]. The question, 'what evolutionary processes have generated chemical diversity in large tropical plant genera?' is thus central to understanding the origin of global insect diversity. Similar evolutionary processes act in other plant genera. But large tropical plant genera (defined as those with >500 species; [11]) represent ideal models as they often include multiple species growing in sympatry that are exposed to the same pool of

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insects. This allows for a comparative framework and direct tests of how trends in host chemistry cascade to other trophic levels and affect their diversity [12].

To date, the evolution of direct constitutive defences (Table 1) has received most attention [7*,9,10*,13,14]. Such defences protect plants against herbivores through toxic or deterrent effects. These compounds can be sequestered by specialized herbivores and used as a protection against predators, thus affecting higher trophic levels [15]. But there is a growing body of evidence that evolutionary trajectories of indirect defences and low-molecular-weight volatiles are also crucial for understanding chemical diversity [8,16**]. Infochemicals (semiochemicals) such as herbivore induced plant volatiles (HIPVs) contribute significantly to plant chemical diversity because their deployment is often species specific [17-19]. Furthermore, indirect induced defences mediate interactions and communication between multiple trophic levels [18*].

Here we identify key evolutionary mechanisms contributing to the diversification of defences in large tropical plant genera, presenting current hypotheses and future directions. Furthermore, we discuss the conceptual and methodological advances necessary for future progress. We highlight the importance of data on associated insect communities, and where possible their phylogenetic relationships, for interpreting these trends.

Evolution of chemical diversity in large tropical plant genera and herbivore diversity

Recent results illustrate that the evolution of host-plant defences can follow various trajectories including escalation, de-escalation, and divergence (Table 2). Both escalation and divergence of defensive traits may contribute to diversification or variation in host-plant defences, which subsequently affects communities of insect herbivores [e.g. 7*,10*,14,20*] (Fig. 1).

Escalation should promote diversification of plant secondary metabolites over the course of the insect-plant arms-race, increasing the species level (α) diversity of secondary metabolites [21]. In theory, if escalating defences are extremely efficient in providing protection against entire communities of herbivores, selection on other chemical traits could be relaxed, leading to a decline in their diversity. However, empirical evidence suggests that even highly toxic or novel defences seldom provide comprehensive protection against all species and guilds of insects [22]. Individual defensive traits thus often show independent evolution as found in *Ficus* or *Inga* [7*,20*]. Escalation in one trait does not necessarily need to lead to de-escalation in another one. However, negative dependence between the diversity of individual metabolite classes can be still expected in the case of negative dependence in metabolic pathways [23].

Escalation of highly toxic defences can help plants to avoid herbivory by generalists, leaving the community dominated by specialists [7*]. This is likely to decrease the overall diversity of the insect community but can promote the adaptive radiation of specialists [21]. Indeed, there are some examples of species rich and specialized herbivore genera associated with toxic plants [e.g. 24]. In contrast, we hypothesise that less toxic defences (e.g. anti-feedants) may lower the community mean for insect performance and facilitate shifts in insect relative abundance, rather than serve to exclude herbivores from the community (Fig. 1). Increased α-diversity of such secondary metabolites may therefore promote insect diversity as found in *Ficus*, possibly by preventing over-dominance of abundant herbivore species [7*].

But herbivores can adapt to predictable defences, using them as feeding cues [25]. This renders such defences largely inefficient against specialized insects [13,26]. Hosts sharing the respective defensive trait can become susceptible and likely to share their fauna of specialized herbivores. This can either lead to de-escalation in defences or push host communities towards divergence as originally suggested for less diverse genera such as *Asclepias* or *Bursera* [13,27].

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The promotion of β-diversity in defences among divergent hosts within large tropical plant genera is likely to increase variation in their insect communities [8,28] (Fig. 1). Shared specialized herbivores can be important selection agents in large tropical plant genera such as *Psychotria*. Congeneric *Psychotria* species show similar habitat preferences and grow in sympatry [4]. Individual species often share up to 50% of their herbivores [29]. Under such conditions, divergence in defences can be expected to be a prominent evolutionary trend, and as a consequence it can reduce the overlap of specialized herbivores among sympatric hosts. Indeed, interspecific differences in chemistry of *Psychotria* species can be of a greater magnitude than intergeneric differences between *Psychotria* and other plant genera [10*]. Similar divergence has been found in many large tropical plant genera such as *Eugenia*, *Ficus*, *Piper* or locally diverse *Inga*, promoting chemical β-diversity between congeners and probably helping them to avoid herbivory [7*,8,10*,30].

The divergence in large tropical plant genera is not limited to direct constitutive defences. The divergence in *Piper* HIPVs has been shown to reduce herbivore inflicted damage to close relatives, likely by either confounding cues for herbivores or through the recruitment of specialized parasitoids (see *Evolution of host-plant defences and its effects on parasitoids* below) [8]. Similarly, there is high variation between individual species of *Inga* not only in their secondary metabolites but also in ant mediated defences [30]. This can potentially further promote the divergence in defensive strategies among closely related *Inga* species. Similar trends can be expected also in *Macaranga* that includes many sympatric species which employ various levels of chemical and ant based defences [31]. Both *Inga* and *Macaranga* include "only" around 300 species but form locally diverse assemblages, forming an important source for insects similar to large tropical genera.

Evolution of host-plant defences and its effects on parasitoids

The effects of plant chemical diversity cascade to parasitoids. For example, *Piper* chemical diversity shapes both caterpillar and parasitic wasp community composition [32*]. This is due to i) the effects of host chemical profile on the susceptibility of herbivores to parasitoids, ii) effects mediated by the changes in herbivore community structure and iii) direct effects of divergence in volatile cues, such as HIPVs (Fig. 1).

First, the chemical composition of an herbivore's diet can directly cascade to parasitoids [33**]. Such cascading effects may be especially strong in the case of the highly specialized communities associated with hosts possessing escalated toxic defences (Fig. 1). Highly specialized herbivores can often sequester host plant metabolites [e.g. 34,35]. Sequestration can protect herbivores from vertebrate predators or disrupt their anti-parasitoid physiological defences, such as melanisation. This can make sequestering herbivores more attractive to insect parasitoids [15,36]. Recent evidence suggests that the resulting effects of host-plant chemical profiles cascading to the third trophic level may show relatively wide geographic variation. For example, higher chemical diversity of *Piper* hosts can facilitate both increased and decreased herbivore resistance to parasitoids [33**]. The studies in *Piper* should be matched by a similar effort in other large tropical plant genera. This may help to further reveal how the interplay of host chemical diversity and herbivore susceptibility to parasitoids is modulated by the life-history of particular species and geographic variation in biotic and abiotic conditions [32*,33**].

Second, hosts with divergent defences, escalated toxic defences, or escalated anti-feedant defences harbour distinct herbivore communities as outlined above [7*]. As many insect parasitoids show relatively high levels of specialization [37], the effects of evolutionary trends in direct constitutive defences can cascade to the third trophic level and promote variation in parasitoid communities as well (Fig. 1).

Third, divergence in indirect induced defences, such as HIPVs, between closely related hosts could allow for recruitment of specific parasitoids. This may further promote variation in parasitoid communities between hosts. The evidence for such patterns is still scarce, although they potentially still play a crucial role [32*]. But we can draw on well-studied pollinator-plant interactions. For example, divergence in volatiles seems to be an important source of variation in pollinating wasp communities in *Ficus* [38]. Host plant volatiles also play a central role in determining pollinator-parasitoid interactions in this system [39]. The scope of current eco-evolutionary studies should be extended to other forms of plant defence in order to examine the effects of their evolution on insect diversity in a tri-trophic context [40]. Methods relying on passive volatile sampling can help considerably here [e.g. 41,42]. They represent an excellent alternative to active sampling methods when exploring volatile profiles in remote tropical regions. These methods mitigate the difficulties associated with transport and electrical supply and allow researchers to run a larger numbers of replicates, as they drastically reduce material costs [41].

Measuring plant chemical diversity has its own pitfalls

Central to interpreting the interplay between evolutionary trends in plant chemical diversity and insect communities are untargeted metabolomic methods [9,43*]. These approaches couple separation and detection methods such as liquid chromatography (LC) with mass spectrometry (MS), or use nuclear magnetic resonance (NMR) spectroscopy without the prior separation of the compounds. The LC-MS approach, for instance, enables structural comparisons among large sets of unknown compounds, computing their structural similarity matrices that are superior to simple diversity measures such as Shannon diversity [43*].

However, it should be noted that all analytical methods have limits in terms of the breadth of compound detection. Metabolomic analyses typically do not optimise extraction on a compound

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group basis, and this may affect both the composition and concentration of detected compounds. For instance, the choice of the extraction solvent alone may cause up to a 10-fold difference in the content of individual metabolites detected [44]. In terms of detection, NMR is more efficient than MS in detecting all types of major metabolites present in the plant extract, while MS is more sensitive and thus more suitable for detecting compounds present in low concentrations. The LC separation of the hundreds of compounds in plant extracts also favours LC-MS over NMR. However, the typical MS metabolomics methods cannot detect (and/or extract) large metabolites such as polymeric tannins. These need a separate analysis protocol that uses UPLC-QqQ-MS/MS for targeted multiple reaction monitoring analysis of four types of tannin groups together with untargeted full scan analysis that allows the detection of many individual tannins [45,46]. To conclude, it is important to always recognise that any single method is not perfect and cannot detect and quantify all defence compounds. In these cases, the quantification of biologically relevant bioactivities may yield another level of information that metabolomics tools miss [e.g. 7*,47].

Linking plant chemical diversity to insect data

Integrating detailed insect community data can help to interpret complex metabolomics data sets. First, obtaining data on herbivores naturally associated with the focal plant lineage allows us to select candidate herbivores for informed manipulative experiments and feeding trials. Indeed, such tests are needed to reveal how metabolite structures may be linked to their activities [48]. Second, having detailed insect data allows identifying metabolites linked to insect community structure. This is key for interpreting evolutionary trends in plant defences since the production of secondary metabolites can be subject to different degrees of selection depending on their function [16**]. Statistical learning regularization techniques, such as

LASSO [49], which can handle complex data can be of great help in identifying the metabolites most strongly involved in the insect-plant interactions [16**].

While we begin to understand the distribution of traits between congeneric hosts and better estimate herbivore pressure [8-10*], herbivore and parasitoid communities remain largely unexplored. So far, many studies have focused on a limited number of insect taxa and guilds, especially caterpillars, and in a bi-trophic context only [e.g. 7*,20*]. This is largely due to the expertise and time needed for the identification of insects in the tropics where delimiting species boundaries is a difficult task. However, a recent renaissance of taxonomy facilitated by DNA barcoding allows us to link refined species to community ecology [50]. Beyond barcoding, molecular data are needed for the robust and informative evolutionary reconstructions central to phylogenetic comparative methods.

A traditional shortcoming has been the relative shortage of statistical methods available for analysing quantitative multivariate community data in a phylogenetic context. But excellent progress is being made in this direction [51,52]. Following these authors, we urge for a tighter inclusion of matched plant and insect community phylogenies that can help understand the evolution of host use and phylogenetic constraints, and to some extent discriminate between higher level macro-evolutionary processes, assigning directionality. For example, densely sampled and well resolved genomic phylogenies of entire insect and plant clades are needed to estimate ancestral plant traits and levels of insect host shifting as well as to provide the context under which plants evolved. Dated phylogenies are particularly relevant here, helping to examine the role of insects themselves in generating chemical diversity and the potential for sequential radiation of insects across plants and cyclical evolutionary dynamics. Large genera with contrasting ages of divergence, such as *Ficus* (ca 75 MYA) or *Inga* (ca 2-10 MYA), are ideal models for such comparative studies on the diversification of insects across plants [7*,20*].

Conclusions

Overall, we are now in a unique situation when novel methods allow us to explore the evolution of various types of defences and their effects on tri-trophic interactions in large tropical plant genera as never before. These plant genera can indeed serve as model systems for studying the genesis of plant and insect diversity - their herbivores can represent up to 30% of local fauna in the case of some herbivore lineages [47]. Studies from individual plants systems have so far excelled in individual aspects: for example in metabolomics, in insect community ecology or in providing the evolutionary context [e.g. 7*,8-10*,20*,30]. Increasingly available metabolomics and genomics methods call for a synthesis and synchronization across all these systems and various geographic regions.

Acknowledgements

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Here the authors investigated chemical structural similarity among closely related trees from several speciose tropical genera. They show that differences in secondary chemistry among closely related congeneric species are large relative not only to within- species variation but also to variation between different genera. Such large differences may in turn permit niche segregation among congeneric tree species based on chemical defence.

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This review provides the most current and comprehensive summary on the role of HIPVs in tritrophic interactions. The authors provide an overview on the current knowledge on the mechanisms involved in forming and mediating HIPV signals. They discuss ecological roles of HIPVs in both natural and agricultural ecosystems. Furthermore, the authors identify the gaps in our understanding of how the HIPVs and their specificity evolve.

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The authors show that host defences are more important for insect host-use than host phylogeny, especially in recently radiated plant genera, such as *Inga*. Furthermore, they suggest that plant defences may be more evolutionarily flexible than insect adaptations to their hosts, allowing plants to persist in the arms race with their insect herbivores.

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This study shows that chemical diversity of *Piper* hosts lowered caterpillar diversity and promoted diversity of the associated parasitoids. The chemical composition of the hosts was highly affected by elevation. This suggests that abiotic factors may play an important role in modulating the effects of plant chemical diversity in the tri-trophic context.

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This study links the chemical diversity in diet of herbivres to their succeptibility to parasitoids. Higher chemical diversity of the hosts significantly affected herbivore immunity and parasitism rates. But the direction of the interaction showed large variability, suggesting that increased chemical diversity can facilitate both increased and decreased herbivore resistance to parasitoids depending on the location and species involved.

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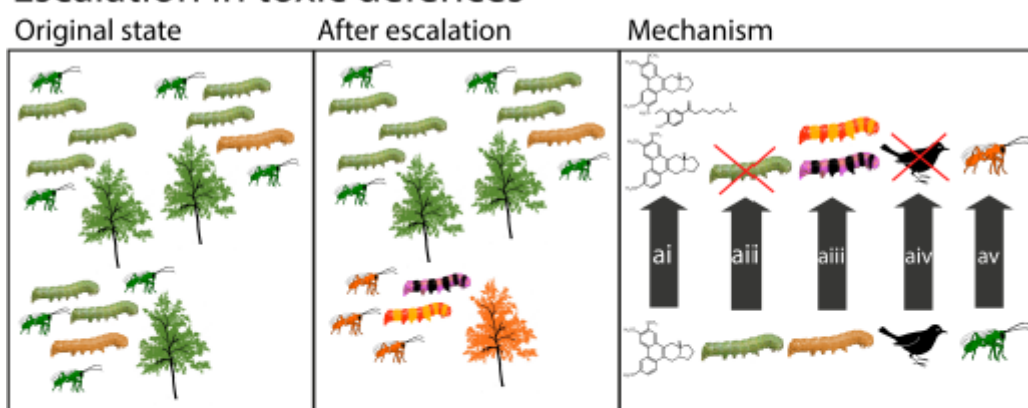
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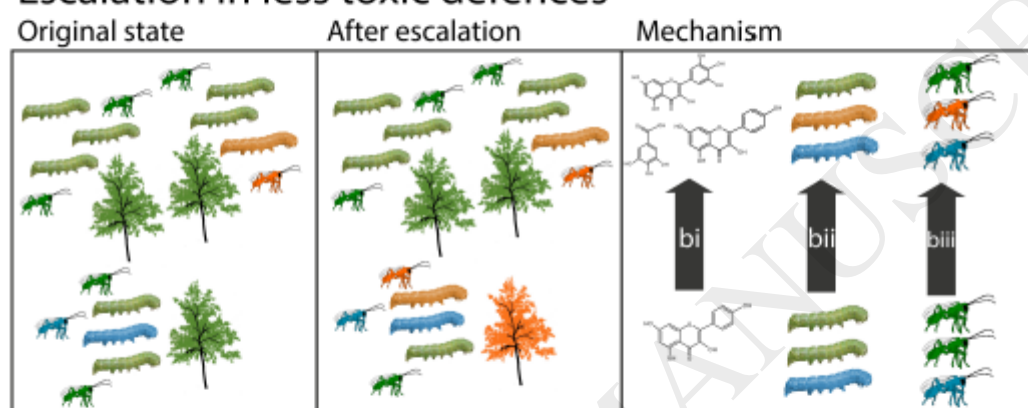
Figure captions

Figure 1. Proposed effects of evolutionary trends in host plant defences on insect diversity. Escalation in toxic defences (ai) frees hosts from herbivory by generalist herbivores (aii). But it can eventually favor speciation of specialized, sequestering herbivores (aiii). The sequestration can lower attractivity of such herbivores to predators (aiv) or disrupts their physiological defences making them preferred by specific parasitoids (av). In contrast, escalation in less toxic defences (bi) probably does not lead to exclusion of herbivores from the community, but rather shifts relative proportion among species. This can increase the diversity of herbivores associated with the respective host (bii) and have a positive cascading effect on parasitoid diversity (biii). A divergence in direct defences (ci) lowers the amount of herbivores the hosts share and promotes herbivore community variation between the hosts (cii). This cascades to parasitoids, promoting variation in their communities as well (ciii). A divergence in HIPVs can further promote the effect (civ).

Escalation in toxic defences
Original state · After escalation · Mechanism

ai · aii · aiii · aiv · av

Escalation in less toxic defences
Original state · After escalation · Mechanism

bi · bii · biii

Divergence
Original state · After divergence · Mechanism

ci · cii · ciii · civ
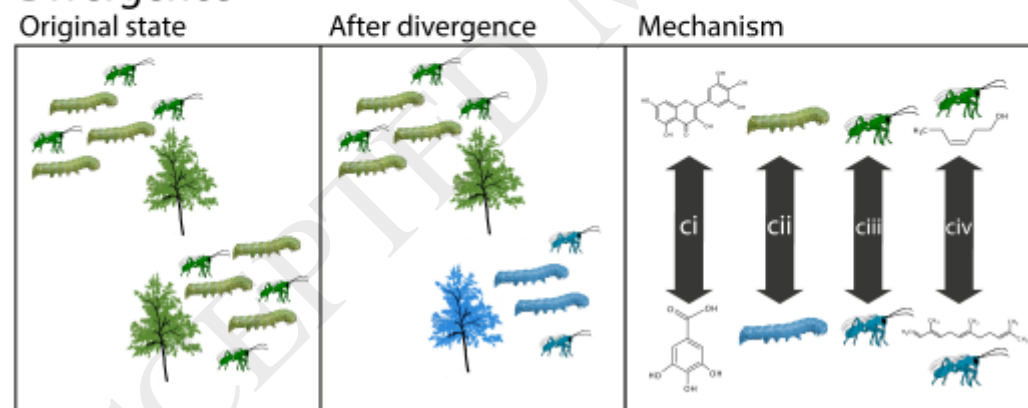
Tables

Table 1. Used terms and definitions.

Term	Definition
Escalation of defences	Macroevolutionary trend towards higher concentration, activity, or diversity of host defences
De-escalation of defences	Macroevolutionary trend towards lower concentration, activity, or diversity of host defences
Divergence in defences	Marcoevolutionary trend towards disparity in defences between closely related hosts
Constitutive defences	Defences that are always present in the plant
Induced defences	Defences that change in their expression or concentration in response to herbivore or pathogen attack
Direct defences	Chemical or physical defences targeted directly at the herbivore, thereby affecting its preference of performance. They can be constitutive and/or inducible.
Indirect defences	Chemical defences attracting predators or parasitoids impacting the herbivores. These include e.g. herbivore induced plant volatiles or extrafloral nectaries. They can be constitutive and/or inducible.
Targeted metabolomics	Metabolomics analysis targeted at identification and quantitation of a defined set of metabolites.
Untargeted metabolomics	Metabolomics analysis focused on quantification of overall metabolome profile and comparing it across samples.

Table 2. Evolutionary trends in defences recovered in plant genera. Large tropical plant genera

with at least 500 species are reported first. Other plant genera in which divergence, escalation,

or de-escalation was found are reported below the horizontal line. In the case of *Protium*, the asterisk indicates that while overall chemical profiles showed divergence, compounds with effect on herbivores showed trends towards directional selection. Question marks indicate cases where the presence of a reported trend is likely. Further confirmation requires additional analyses and/or wider sampling effort.

	Number of species	Divergence in defences	Escalation of defences	De-escalation of defences	References
Eugenia	~1,000	?			[10*]
Ficus	~800	yes	yes		[7*]
Piper	>1,000	yes			[8,9]
Psychotria	~1,850	yes			[10*]
Solanum	>1500	?			[53]
Asclepias	>140		yes	yes	[13,23]
Bursera	~100	yes	yes		[14,27]
Inga	~300	yes			[20*,30]
Ocotea	>300	?			[10*]
Protium	~150	yes*			[16**]
Salix	~400			?	[22,26]
Streptanthus	~35			yes	[54]