Ph.D. Thesis review by Prof Owen T. Lewis, University of Oxford, UK.
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Thesis: **Specificity of insect-plant associations and their role in the formation of plant defenses and speciation**
Candidate: RNDr. Martin Volf

Overview
The thesis investigates the role of interactions between insect herbivores and their host plants in the evolution of plant defenses, and the consequences for diversification of both plants and insect herbivores.

Overall, the thesis is comprehensive, well-reasoned and gives a clear account of the work conducted. Each data chapter is thorough and the thesis is very well-written, with English of a high quality and with few typographical errors. Methods and strategies of statistical analysis are particularly well described and justified throughout, and the results are discussed and interpreted in considerable detail. The candidate's ability to compile, analyze and interpret large datasets is ably demonstrated, and a variety of different approaches had been mastered including fieldwork, species sorting and identification, molecular approaches to species delimitation and identification, and advanced phylogenetics methods. The candidate also demonstrates skilled and appropriate application and interpretation of a variety of sophisticated univariate and multivariate statistical analyses.

Particular aspects of the thesis that represent substantial and important contributions to the literature, and which will be of interest to a wide audience. These include the discovery of species-specific and taxon-specific responses of insects to defensive compounds in their host plants. This may drive plant defenses to diversify in a way that provides protection against multiple specialised herbivores. The thesis also generates novel data on how plant phylogenies might shape the foodwebs of insect herbivores associated with them, revealing contrasting effects for specialised and less-specialised insects.

Detail of individual chapters
Of the five data chapters, all are written in paper format. Two are already published in leading international journals, and the remainder will also be publishable is good journals with little modification from their current form.

Introduction & Summary
The Introduction provides a concise but authoritative and up-to-date survey of the field of plant-herbivore interactions and defense. It sets the scene for the data chapters, indicating clearly the gaps in knowledge that are to be addressed and how the component chapters fit together. The Summary is rather brief and (given the paper format of individual data chapters) it might have been helpful to have a more wide-ranging general discussion to allow a more thorough synthesis of the results of the different chapters.

Chapter 1 - *To each its own: differential response of specialist and generalist herbivores to plant defence in willows.*
This chapter has already been published (2015) in *Journal of Animal Ecology*, one of the top journals in the field. It examines whether leaf-chewing insects (both specialists and generalists, from three different orders: Coleoptera, Lepidoptera and Hymenoptera) respond in a consistent way to plant phylogeny, nutrients, and both chemical and mechanical

defenses. The chapter is novel in investigating a species-rich assemblage exploiting a clade of related plants, rather than being focused more narrowly on individual pairs of interacting species. Data were collected from field sites in the Czech Republic and the chapter involved fieldwork, chemical analyses and phylogenetic reconstruction, as well as a range of sophisticated statistical analyses. Plant chemistry (specifically, secondary metabolites) and physical defenses (trichomes) were both shown to influence associations with generalist and specialist insect herbivores, but with important differences in the response of the two herbivore groups. These variable patterns may prevent plants from evolving "universal" anti-herbivore defenses and encourage diversification of defensive traits. I did not identify significant weaknesses or omissions in this excellent chapter.
Questions:

1. How typical is *Salix* as a focal taxon? Its chemistry is well-studied but is it particularly well defended in comparison with other temperate shrub and tree genera?
2. Would you expect patterns in plant defenses (and insect responses to them) to be similar in short-lived herbaceous plants?

Chapter 2 - *Insect herbivores drive the loss of unique chemical defense in willows.*
This chapter has already been published (2015) in *Entomologia Experimentalis et Applicata.* The paper builds on the insect-*Salix* study system investigated in Chapter 1, investigating whether the protective benefits of novel defensive traits lead to their diversification. Although high content of salicylate defensive metabolites was associated with low herbivore diversity and high host specificity, *Salix* phylogenies reveal that salicylates have actually been lost in some *Salix* species, rather than diversifying further as might be expected. The chapter provides a sensible and well-argued attempt to resolve this apparent paradox, focusing on the balance of costs and benefits.
Questions:

1. Is it valid to divide willow species into two groups (high and low salicylate concentration) for analysis? Given that salicylates are measured on a continuous scale, and there is a 6-fold variation in concentrations within the 'high' category, would analyzing salicylates as a continuous variable be potentially informative?
2. What can you say about *intra*specific variation in salicylates – is it small relative to interspecific variation, and does it seem to have an impact on associated insects?

Chapter 3 - *Dynamic plant defenses of sympatric* Ficus *species structure local larval leaf-chewer communities*
In this chapter, an impressive dataset is assembled for a diverse, co-occurring set of 21 *Ficus* species from field sites in Papua New Guinea. The chapter investigates insect herbivore communities in relation to *Ficus* phylogeny and plant chemistry. The data provide a valuable extension and comparison to the *Salix* data presented in Chapters 1 and 2. The results are fairly complex and challenging to summarise concisely. Overall, different defensive traits are shown to follow different evolutionary trajectories, rather than a pattern of uniform divergence or uniform diversification. From the insect perspective, community structure was significantly affected by these traits, with specialised and less-specialised herbivores responding in different ways to different plant traits (as in the *Salix* system). The chapter interprets these results intelligently and logically.
Questions:

1. Why limit the analysis to a subset of the larval leaf chewer community, when wider data are available for other insect herbivores? Would you expect other insect herbivore guilds to show similar patterns?

Chapter 4 - *Phylogenetic diversity of host plants drives insect- plant food web structure.* This chapter investigates how host plant diversity effects insect specialisation at three temperate sites in the Czech Republic and Japan. This chapter involves a spectacular amount of fieldwork and the effort that has gone into assembling these datasets should not be underestimated. The novelty of the approach used is that it helps to reveal the stages during plant radiations that are responsible for generating insect diversification. The results reveal a strong impact of host plant phylogeny on the structure of insect food webs, with different insect lineages responding to plant diversity at different levels in the phylogeny.
Questions/ Comments:

1. Can you elaborate on the two measures of specialisation analysed (generality and H_2'). How do they differ and is information from them complementary?
2. There will be many 'historical' plant lineages missing from 'modern' plant phylogenies at these sites. Furthermore, many of the insects may be relatively recent colonists in the particular sites rather than having a long-term shared evolutionary history in these locations. How does this affect the interpretation of the results?
3. [There is an unfortunate typographical error ('specious' instead of 'speciose') in the first sentence of the Introduction].

Chapter 5 - Speciation in a keystone plant genus is driven by elevation: a case study in New Guinean *Ficus*.
Here population genetic structure is investigated for two species of *Ficus* along an extensive elevational gradient in Papua New Guinea, revealing strong barriers to gene flow separating lowland and highland conspecific populations. Spatial population genetic structure differs markedly for the two populations, with lowland subpopulations being panmictic over a much larger spatial scale. It is suggested that mountain subpoulations may be restricted by reduced pollen and seed dispersal, with local adaptation possibly contributing. There are important implications for diversification and also potentially for biotic responses to climate change.
Questions/Comments:

1. The chapter includes *Ficus* community diversity data as well as within-species population genetic data for two species. The community focus was not really anticipated in the Introduction or integrated in the Discussion, and it was not clear to me how well these two datasets fitted together and the rationale for studying them together.
2. Are these results relevant in understanding biotic responses to climate change, given that species may need to shift in elevation as the climate warms to remain within a favourable bioclimatic 'envelope'?

Recommendation
In summary, in my opinion the thesis represents a substantial and original contribution to scholarship. My recommendation is therefore that the thesis is worthy of the award of PhD.

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Review on the PhD thesis of Martin Volf
„Specificity of insect-plant associations and their role in the formation of plant defenses and speciation"

This thesis has an introduction and five chapters represented by published papers or manuscript, and since two of the chapters have been published in good journals, there is no doubt that the thesis fullfils all the necessary criteria. The topic of the thesis is interesting and the results are convincing. Hereafter I will comment on individual parts of the thesis (the questions for Martin are in italics).

Introduction is well written in terms of the language and clarity of formulations, but is a bit messy as it aims to cover quite disparate topics, and it is not clear what genre it is. It is too unfocused for a proper review, and it does not adress in detail the content of the thesis to be a real introduction to the chapters which follow, so it is something between. Also, the last part concerning figs, their pollination and diversification, seems a bit unrelated to the previous topics. I have one general question: the author claims that there is no doubt that high diversity of host-plants is one of the key factors maintaining hot-spots of exant insect diversity. *But do we really have an evidence for it?* An alternative view would be that there are some other reasons for insect diversification (e.g. high temperature promoting speciation and stable environment lowering extinction), and the specialization of insects to individual host plants (whose diversification could have similar reasons) is just a secondary effect utilizing the diversity of potential hosts. *Is there a way how to reject such a hypothesis?*

Chapter 1 comprises differential responses of specialist and generalist herbivores to plant defense in willows, showing that plant defense needs to be studied with entire insect assemblages. It is an important message, and since this part has been published in a very good journal, it does not make much sense to comment on it. The same applies for **Chapter 2** which shows that the evolution of plant defenses may be complex, including even loss of some of the defensive traits. The study elegantly shows that while plant defense based on salicylates may affect total insect diversity, it may not be reflected in insect abundance, so that this defensive trait may be in the end not that advantageous.

Chapter 3 is an unpublished manuscript dealing with various defensive traits and their effects on several insect guilds. It is very comprehensive and complex, which is also its major weakness – the findings are so diverse that it is difficult to take a clear picture about what is going on there, besides the feeling that the evolution of plant defenses and its coevolution with different types of insects is much more complex than we could ever imagine. I think it would be good to make the results clearer, e.g. dividing them into some meaningful groups and pointing out the most important results; otherwise it is pretty difficult to read. Also, I am not sure that the methods used have been those most appropriate. *Since the matrices of traits, species, and local communities were analyses, why the authors have not used some verison of the fourth-corner analysis instead of dealing with mean values?* I have also two more general questions. First, Martin mentions at the end of the first page of the Introduction section that it is possible that different evolutionary processes act across large and local scales among closely related plant species. *But what would be these different processes and how it could work?* This should be clarified. Second, *given the disparity and complexity of the results, is it in principle possible that the evolution of plant defense is actually a sort of neutral process?* I could imagine that - given the multitude of defensive traits aimed towards various natural enemies and the complexity of herbivore communities - an acquiring a novel defensive trait may never represent a substantial advantage, and its fixation then could be a matter of chance rather than representing a predictable adaptive progress. Of course, it must represent some incremental advantage at the beginning to be fixed, but this may be latter on balanced by coevolution with both the insect and plant competitors, so that in the end it may actually not matter too much which of the traits has been acquired in any particular step.

Chapter 4 concerns the role of various phylogenetic depth on the inferred insect specialization for different guilds. It is much clearer than the previous chapter, and the results are sound and relatively straightforward. I have only a couple of technical comments. Most importantly, the authors speak about „vulnerability“ in the Results section, although this measure has not been introduced yet in the Methods, and in fact it is not clear at all what it is. Also, I am wondering why the bars representing abundance of individual host plants in fig. 1 differ in their length between studied insect guilds, although it is still the same plant community. For instance, *why is C. bet much longer in the middle row of the last column (Mikulcice) than in the bottom row*? More generally, *is the number of evolutionary lineages in Czech localities sufficient for such analyses?* The results are interesting, but I worry that the differences in the patterns between the localities may be ultimately driven just by the fact that only very few tree species live in central Europe, and they are phylogenetically relatively distant.

Chapter 5 deals with genetic differentiation of two fig species along an elevational gradient. It is very well written and clear, the results are convincing and interesting, though somehow unrelated to the main topic of the thesis. I only feel that the text (especially Introduction section) is a bit unclear in whether it refers to the differentiation along the gradient or the differentiation of high-elevation populations from each other due to the fact that these populations are somehow separated from the lowland populations (probably both, but these two effects should have been distinguished). Also, I wonder why the authors stress the role of the above-canopy winds in dispersal (or its absence), given that they study understory tree species. *Is there any reason to assume that wind would play a role in understory species?*

David Storch