Peer Community In Evolutionary Biology

Genetic intimacy of filamentous viruses and endoparasitoid wasps

Ignacio Bravo *based on peer reviews by* ***Alejandro Manzano Marín*** *and 1 anonymous reviewer*

D. Di Giovanni, D. Lepetit, M. Boulesteix, M. Ravallec, J. Varaldi (2018) A behavior-manipulating virus relative as a source of adaptive genes for parasitoid wasps. Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Evolutionary Biology. https://doi.org/10.1101/342758

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Viruses establish intimate relationships with the cells they infect. The virocell is a novel entity, different from the original host cell and beyond the mere combination of viral and cellular genetic material. In these close encounters, viral and cellular genomes often hybridise, combine, recombine, merge and excise. Such chemical promiscuity leaves genomics scars that can be passed on to descent, in the form of deletions or duplications and, importantly, insertions and back and forth exchange of genetic material between viruses and their hosts. In this preprint [1], Di Giovanni and coworkers report the identification of 13 genes present in the extant genomes of members of the *Leptopilina* wasp genus, bearing sound signatures of having been horizontally acquired from an ancestral virus. Importantly the authors identify *Leptopilina boulardi filamentous virus* (LbFV) as an extant relative of the ancestral virus that served as donor for the thirteen horizontally transferred genes. While pinpointing genes with a likely possible viral origin in eukaryotic genomes is only relatively rare, identifying an extant viral lineage related to the ancestral virus that continues to infect an extant relative of the ancestral host is remarkable. But the amazing evolutionary history of the *Leptopilina* hosts and these filamentous viruses goes beyond this shared genes. These wasps are endoparasitoids of *Drosophila* larvae, the female wasp laying the eggs inside the larvae and simultaneously injecting venom that hinders the immune response. The composition of the venoms is complex, varies between wasp species and also between individuals within a species, but a central component of all these venoms are spiked structures that vary in morphology, symmetry and size, often referred to as virus-like particles (VLPs). In this preprint, the authors convincingly show that the expression pattern in the *Leptopilina* wasps of the thirteen genes identified to have been horizontally acquired from the LbFV ancestor coincides with that of the production of VLPs in the female wasp venom gland. Based on this spatio-temporal match, the authors propose that these

VLPs have a viral origin. The data presented in this preprint will undoubtedly stimulate further research on the composition, function, origin, evolution and diversity of these VLP structures, which are highly debated (see for instance [2] and [3]).

References:

[1] Di Giovanni, D., Lepetit, D., Boulesteix, M., Ravallec, M., & Varaldi, J. (2018). A behavior-manipulating virus relative as a source of adaptive genes for parasitoid wasps. bioRxiv, 342758, ver. 5 peer-reviewed and recommended by PCI Evol Biol. doi: [10.1101/342758](https://dx.doi.org/ 10.1101/342758)

[2] Poirié, M., Colinet, D., & Gatti, J. L. (2014). Insights into function and evolution of parasitoid wasp venoms. Current Opinion in Insect Science, 6, 52-60. doi: [10.1016/j.cois.2014.10.004](https://dx.doi.org/ 10.1016/j.cois.2014.10.004)

[3] Heavner, M. E., Ramroop, J., Gueguen, G., Ramrattan, G., Dolios, G., Scarpati, M., ... & Govind, S. (2017). Novel organelles with elements of bacterial and eukaryotic secretion systems weaponize parasites of *Drosophila*. Current Biology, 27(18), 2869-2877. doi: [10.1016/j.cub.2017.08.019](https://dx.doi.org/ 10.1016/j.cub.2017.08.019)

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/342758
Version of the preprint: 2

Authors' reply, 29 November 2018

Download author's reply

Decision by Ignacio Bravo, posted 23 November 2018

Invitation to revise your preprint before recommendation in PCI

Dear Dr. Varaldi

thank you very much for having submitted the revised version of your text to PCI Evol Biol. The same two reviewers that evaluated your text in first instance have provided feedback on the resubmitted one. As you can see in the accompanying reviews, both reviewers acknowlege that you have integrated a large number of their concerns, and I agree with them. Nevertheless, also both reviewers find that some of the points raised have not been properly addressed. I share also this view in what regards to the verbal argumentation of the consistence with the main hypothesis of partial trees that do not present all terminal taxa. I think this is a view that needs be substantiated on quantitative terms rather than on verbal ones. Also, the reviewers mention some instances in the text in which the wording may suggest that you have evidence to sustain a claim for the physical existence of VLPs, that need be substantiated.

Overall, I think that the comments raised by the reviewers can be properly addressed after minor changes to the text, and I think that if you respond in detail point-to-point to each of the criticisms, a third round of peer-review should not be needed.

I sincerely thank you for supporting PCI, and look forward to read your response to the reviewers and the revised version of your text.

Sincerely

Ignacio Bravo

Reviewed by Alejandro Manzano Marín, 05 October 2018

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Reviewed by anonymous reviewer 1, 05 October 2018

The authors have made several revisions based on my comments. The authors agree that a viral origin does not discount the potential status as an organelle; however, this agreement is not reflected within the paper. An example of this is in the following paragraph:

Because 323 the proteins wrapped within the VLPs have a eukaryotic origin and because 324 neither viral transcripts nor viral proteins had been identi_ed from venom 325 gland analysis, it has been claimed that VLPs do not have a viral origin [56], 326 and thus other denomination has been proposed in lieu of VLP [29]. On the 327 contrary, our data strongly suggest that the VLPs found in Leptopilina do 328 have a viral origin and derive from a massive endogenization event involving 329 a virus related to an ancestor of the behaviour manipulating virus LbFV(Fig 330 2B).

This sentence is puzzling given the fact that the authors contend in their response document: "Nowadays, VLPs are eukaryotic structures (organelles) even if some of the key genes involved in their production derive from virus genes."

Taken together, these statements are confusing. Origin of VLPs is discussed without a clear description of our current knowledge of VLPs from various Leptopilina species. Have VLPs been described from the Leptopilina species studied here? What proteins are present in VLPs and do the results in this paper have anything in common with any described VLP proteins? Even a negative result would be worth stating and discussing.

Similarly, in line 401, the authors write: "All together, our data show that VLP production is possible thanks to the domestication of 13 virally-derived genes, captured from an ancestor of LbFV."

Thus, it is clear, the authors are convinced of their idea, even though they have stepped back (superficially) by changing the title of their paper.

In this reviewer's view, the authors have not shown that the 13 virally-derived genes in the Leptopilina genomes studied are involved in VLP production. Their data demonstrate the existence of these genes in wasp genomes and their spatial and temporal expression in venom gland extracts.

As such, these results do not link LbFV genes to venom production, VLP production, or venom/VLP function. It is commendable that the team tried RNA interference experiments. However, in the absence of such results, it is advisable to wait to get the necessary evidence that will shed light on the function(s) of these interesting wasp genes/proteins. Only two sequences have any sequence similarities, but no further experimental data on these or any of the wasp LbFV proteins is available. Thus, there is a significant gap between evidence and interpretation.

Ref 56 has interesting ideas that differ from the ones proposed by the authors. It is worth stating them clearly with underlying evidence. Instead of taking an oppositional view (as in lines 320-330), a more balanced view of pertinent ideas would improve this manuscript and benefit the quality of discussions in this growing field.

Other comments:

(1) I do not understand the reluctance to show alignment of wasp ORFs with viral ORFs. This information would be informative in understanding, for example, where the primers (for expression and copy number studies) bind.

(2) If you have done experiments to check the copy number of shake and actin (used as controls for copy number), provide your evaluation of their copy number in the supplement. In the same context, provide appropriate citations showing that these genes are single copy genes in related genomes.

(3) Regarding the eukaryotic origin of LbGAP, the reference cited is incorrect. Ref. 17 is the correct reference for this. Please make sure all statements are correctly corroborated with appropriate references.

(4) The species for the Ganaspis wasps is changed in this revision. Identification of these wasps is quite difficult. So it is important to say how verification of species used was carried out. Please do this for all species. What criteria were used? Looks like G.x (line 525)—is carried over from the previous version of the paper? Please correct this.

(5) Figure S1 legend: last sentence requires a full stop at the end. Dr. Shubha Govind's name is misspelled in the acknowledgements. Please review the paper for similar errors.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/342758

Authors' reply, 05 October 2018

Download author's reply

Decision by Ignacio Bravo, posted 05 October 2018

Revise

Dear author

thank you very much for submitting your preprint to the open PCI review process. As you can see in the accompanying files, two experts in the field have provided feedback on your submission. Both of them agree on the importance of the question and on the pertinence of the approaches used to tackle it, and I largely agree with them. Also both reviewers have invested a considerable amount of time and energy in providing a detailed report on the manuscript, with an overall very positive judgement on the methods and approaches. Notwithstanding, also both reviewers identify a number of flaws in the text that prevent recommendation in its present state, essentially centered in the , and I also largely agree with them. As you can see in their reviews, some concerns have been raised about the logical flow between results and interpretation. This is the case for instance for the support for the HGT event in the case of all genes depicted in figure 3; for the pertinence of the dn/ds values of conserved arthropod genes used to serve as reference for a set of genes only present in a subtree of these species. In other cases the questions are rather the pertinence and clarity of the figures used, as in fig 1 and fig 2. Finally, I would appreciate if you could include an assessment of the identity of the specimens actually used for the analyses, specially for the case of Ganaspis xanthopoda, as no RNA sequences were available in the screened databases.

Overall, the reviewers and myself are very supportive for recommendation upon revision. I would thus encourage you to respond to each and every point raised in the reviews. In case you think a verbose answer suffices for certain points, I would appreciate if you could make a clear case of scientific cost-benefit evaluation to justify that no novel data generation or analyses are needed.

I look very much forward to reading the revised version of the manuscript.

Faithfully

Ignacio Bravo

Reviewed by Alejandro Manzano Marín, 17 August 2018

Download the review

Reviewed by anonymous reviewer 1, 17 August 2018

This paper identifies some homologs of the behavior-modifying LbFV genes in genomic sequences of L. boulardi (where LbFV is found) and those of L. heterotoma and L. clavipes. It also addresses the possible relationship of these homologs with virulence functions of L. boulardi VLPs. The paper hypothesizes that before diversification of Figitids, LbFV captured 3 insect genes. LbFV is a descendant of this virus that then integrated into genomes of ancestral Leptopilina spp. but after its divergence from Ganaspis. The authors further claim that these integrated viral-like ORFs play a permissive role in generating the immune-suppressive Leptopilina VLPs. According to this scenario, these Leptopilina immune-suppressive VLPs are derived from erstwhile viral genes, now domesticated in wasp genomes. I have the following feedaback: Overall critique:

(1) The authors claim that the expression of the viral-like wasp genes is somehow linked to the expression of the VLP proteins but the details of this linkage are not established. No structural or functional assays establish this proposed relationship of the viral-like wasp genes with VLPs. For example, the Poirie lab has shown that RNA interference-mediated gene knockdown is possible in L. boulardi. Such an approach here would help validate if expression of the viral-like wasp genes is needed for VLP production or their function. In the absence of such functional assays, the main conclusion in the study is not supported and the authors should consider rephrasing parts of the paper, including the title.

In this context, it is important that the authors limit their interpretations for results backed by experimental data in only the wasp species for which experimental data are presented and not generalize the results to species not studied. In many places, the results are over-interpreted.

(2) Copy number experiments: It is well known that cells of the long gland portion of the venom gland cells are endopolyploid. VLP proteins are thought to be produced in these cells. I wonder if it is possible that even at the earliest stages of venom gland development, some venom gland cells undergo endopolyploidy and this affects the copy number differences observed in males and venom gland tissues. The cell type(s) in which copy number amplification is proposed to occur has not been identified. This potential difference (or change) in overall ploidy in experimental and control samples adds a wrinkle in the interpretation of the copy number data.

(3) Real time PCR experiments: The authors have previously shown that LbFV can be found in the oviduct as well as in the venom gland. It is therefore important for them show in control experiments that for the template samples used in the qPCR experiments, there is contaminating material from ovaries or related organs such as the oviduct where the viral-like wasp genes may also be expressed.

(4) Is it possible that VLPs have a viral past but the structures produced by Leptopilina wasps are not viral?

Detailed review:

- Lines 92-93: As stated above, this evidence in L. boulardi (let alone all Leptopilina wasp species) is lacking. Use of the word “permit” raises mechanistic questions for which there is no evidence or discussion.
- Line 134: Of the 17 viral proteins with significant hits in the wasp genomes: what else is known about them. A Multiple Sequence Alignment of FV genes/proteins found in the wasp genomes would highlight the dN/dS statistics they present in Figure 4 as well as introduce the predicted domains in some of these proteins where such homology exists. Are some of these domains exclusively viral or are these domains also present in eukaryotic proteins. It is important for the reader to have this information organized in a cohesive manner at the outset.

-Do viral-like genes in the Leptopilina genomes have introns? I missed this information if it is in the paper. Clarification of these points is important to understanding the hypothesis.

- Regarding proteins 27 and 66 (inhibitors of apoptosis) and 11 and 13 (the predicted methyl transferases): are there eukaryotic homologs in the sequenced Leptopilina and Ganaspis wasp genomes?
- Lines 149-150: The sentence is logically incorrect. Please restate referring to the 13 genes encoding the proteins.
- Make a new paragraph at line 160. In the lines that follow, a new question is raised: is the depth and the GC content of scaffolds of wasp genomes with BUSCO genes and “viral-like” genes versus scaffolds with

viral/bacterial genes similar or different? For the non-specialist, explain why these parameters should be similar or different in these scaffolds? This entire section is confusing and should be restructured and revised for clarity. The limitations of the results should be stated. For example, in line 177, the authors claim that their statistics "demonstrate" the presence of viral-like genes. The data are suggestive and require experimental confirmation (e.g., in situ hybridizations with appropriate probes) to actually demonstrate this. Line 180 is particularly unclear.

-Fig. 3. The data in this Figure constitute the key observation of the paper. It would be great to have experimental evidence to support the predictions of these assemblies in any one of the wasps. Otherwise, they remain predictive and should be stated as such.

Molecular data showing the importance of these ORFs would validate the prediction and importance.

-Lines 252, 614 and other places. Please correct the spelling of actin.

Feedback on figures:

Fig. 1: Show in landscape . - Fig and legend 2: This Figure needs to be reworked with two clear parts (A) and (B). The legend should also be more fully developed.

-Say something about the 3 insect genes in the legend.

Sentence starting: Nowadays, all Leptopilina species bear 13 LbFV-derived... Qualify this sentence to limit only those species that were studied. Has the requirement for the 13 LbFV genes for VLP production been shown for all Leptopilina species or is this a prediction/extrapolation? If it is an extrapolation based on the PCR results of ORF 96 in the other Leptopilina species (Fig S3A), the authors should still confirm that those tested do have the rest of the expected sequences. What was the rationale for studying ORF 96 in detail?

Fig. 2: Are there any data from L. victoriae?

Fig. 2: Remove VLP panels and cite original papers that show the presence of VLPs in these species, unless these panels represent new data; if so, show them more clearly with scale bars and labeling to show VLP morphologies.

Fig. 4: Expand X axis. Words on top of the bars are not readable given the size of the graph. This should be fixed.

Fig. 5: For the light microscopy panels, make the notations and the scale bars clearer. Scale bars need to be inserted in the electron micrographs. Also, what is being observed in these micrographs is not clear. The regions need to be labeled; point to the areas with VLPs and show these areas at higher resolution and magnification to make the observation more convincing.

Fig. 7: Add L. boulardi to the legend.

Feedback on the Methods section:

- The paper states that the L. boulardi genome was deduced from a female infected with LbFV. Were the wasps used in all other experiments were infected or uninfected?
- For copy number experiments, can the primer target sequences viral and their genomic counterparts?
- Line 500, they do not state how they tested for LbFV DNA in L. heterotoma or G. xanthopoda.

-In the Methods section (lines 526-543) discuss the issue of genome sizes. However, they do not reveal if their estimates are consistent with the published work.

-How many actin genes (and how many copies of each) are predicted in the L. boulardi genome and which of these is used to control real-time PCR data? Is its expression the same in males and females?

- Explain what the single copy gene shake encodes? How do you know it is a single copy gene in these wasps?

-How did the authors determine that the RhoGAP is part of the L. boulardi VLP and is not just a protein that is part of the fluid component of the venom?