Peer Community In Infections

Changes in aggressiveness in pathotypes of wheat leaf rust

Pierre Gladieux *based on peer reviews by 2 anonymous reviewers*

Cécilia FONTYN, Kevin JG MEYER, Anne-Lise BOIXEL, Ghislain DELESTRE, Emma PIAGET, Corentin PICARD, Frédéric SUFFERT, Thierry C MARCEL, Henriette GOYEAU (2022) Evolution within a given virulence phenotype (pathotype) is driven by changes in aggressiveness: a case study of French wheat leaf rust populations. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Infections.
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Understanding the ecological and evolutionary factors underlying the spread of new fungal pathogen populations can inform the development of more effective management strategies. In plant pathology, pathogenicity is generally presented as having two components: 'virulence' (qualitative pathogenicity) and aggressiveness (quantitative pathogenicity). Changes in virulence in response to the deployment of new resistant varieties are a major driver of the spread of new populations (called pathotypes, or races) in modern agrosystems, and the genomic (i.e. proximal) and eco-evolutionary (i.e. ultimate) factors underlying these changes are well-documented [1,2,3]. By contrast, the role of changes in aggressiveness in the spread of pathotypes remains little known [4].

The study by Cécilia Fontyn and collaborators [5] set out to characterize changes in aggressiveness for isolates of two pathotypes of the wheat leaf rust (*Puccinia triticina*) that have been dominant in France during the 2005-2016 period. Isolates were genetically characterized using multilocus microsatellite typing and phenotypically characterized for three components of aggressiveness on wheat varieties: infection efficiency, latency period, and sporulation capacity. Using experiments that represent quite a remarkable amount of work and effort, Fontyn et al. showed that each dominant pathotype consisted of several genotypes, including common genotypes whose frequency changed over time. For each pathotype, the genotypes that were more common initially were replaced by a more aggressive genotype. Together, these results show that changes in the genetic composition of populations of fungal plant pathogens can be associated with, and may be caused by, changes in the quantitative components of pathogenicity. This study also illustrates how extensive, decade-long monitoring of fungal pathogen populations, such as the one conducted for wheat leaf rust in

France, represents a very valuable resource for research.

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Reviews

Evaluation round #1

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Version of the preprint: 2

Authors' reply, 02 March 2023

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Decision by Pierre Gladieux, posted 01 December 2022, validated 01 December 2022

Dear Dr. Marcel, Dear all,

Your preprint entitled "Evolution within a given virulence phenotype (pathotype) is driven by changes in aggressiveness: a case study of French wheat leaf rust populations,» which you submitted to PCI-Infections, has been reviewed. The comments of the reviewers are included at the bottom of this letter.

I enjoyed reading your manuscript, which represents a large amount of experimental work and presents interesting and insightful results. However, based on the comments of the reviewers and my assessment, your manuscript will require revisions before further consideration for a recommendation.

One main concern raised by reviewer 1 is the nature and presentation of statistical analyses. Please review your analyzes and their presentation and modify your conclusions accordingly.

Another concern raised by reviewer 1 is the content and length of the discussion. Four pages of results and ten pages of discussion seem highly unbalanced indeed.

Both reviewers and I (see below) also expressed various concerns regarding the hypothesis at the core of this study. After reading your preprint, I have the impression that you are not testing the hypothesis described in the abstract or introduction but another, which also has its merits but needs to be better introduced.

The central hypothesis of the article is unclear for several reasons:

- The introduction suggests that changes in aggressiveness are caused by quantitative resistance. For instance, line 102 reads: « the quantitative resistance genes introgressed into new wheat cultivars at the same time as qualitative resistance genes may also exert a selection pressure in favor of more aggressive pathotypes .» However, as underlined by both reviewers, the connection between natural selection for increased aggressiveness and quantitative resistance of the host is unclear: do we need to invoke quantitative resistance to explain the evolution of aggressiveness? Then, if the hypothesis is that quantitative resistance is at the origin of the variations of aggressiveness, why never mention - and test the effect of - the variations of quantitative resistance between the hosts included in the annual survey and the experiments? Finally, this sentence also conveys the idea that the genetic bases of quantitative and qualitative resistance would be distinct, just like the genetic bases of virulence or aggressiveness, which is probably an unnecessary simplification, as pointed out by reviewer 2.
- As I struggled to understand your hypothesis, I read Fontyn et al. 2022 doi.org/10.1111/ppa.13599. In this article, if I understand correctly, you formulate the hypothesis according to which the pathotypes 166 317 0 and 106 314 0 correspond to generalist lineages, whose greater aggressiveness allows them to outcompete several other compatible pathotypes virulent against the Lr genes carried by the most widely grown cultivars (9th paragraph of the discussion, starting with « PCA revealed… »). You take up this hypothesis in the introduction to your preprint L112-118. Based on this hypothesis, I expected to see a comparison between the major pathotypes and the other pathotypes. Instead, I observed that you were interested in the genotypic diversity of pathotypes and that you tested the hypothesis of a role of aggressiveness in the temporal dynamics of genotypes underlying the same pathotype.

I have highlighted some reviewer comments here, but I invite you to consider each in your revised preprint.

Other comments:

L132 and L232-236: you used five cultivars widely grown over the study period. How many resistance genes matching virulence in the pathotypes do each of these cultivars carry?

L235: « were among the 35 most frequently grown cultivars in the French landscape during the 2006-2016 period. ». How frequent are they? What is the frequency of the 35th most frequently grown cultivar?

Reviewed by anonymous reviewer 1, 17 November 2022

In this manuscript, the authors argue that the evolution of virulence in the French wheat leaf rust population was driven by changes in the aggressiveness of the pathogen. They employed relevant experiments to achieve the goals of the study, used appropriate experimental designs and statistical procedures to analyze the data and interpret the results, and wrote the paper well.

In the introduction section and throughout the manuscript, the authors clearly defined the difference between virulence and aggressiveness. However, I would tend to disagree that virulence is purely qualitative and that the function of Avr proteins is solely for recognition by the plant R proteins. Several recent studies on rusts and other pathogens revealed that Avr proteins or 'effectors' have diverse functions, including blocking and manipulating host defenses. Any of them can be recognized by plant R proteins, which would trigger a downstream signaling response leading to effector-triggered immunity. In this sense, I view virulence in a more quantitative perspective and believe that the aggressiveness phenotypes described in the study are just manifestations of the other functions of effectors. In flax rust and oat crown rust, hundreds of predicted effectors were identified and they could have different functions.

In the methods section, the authors conducted intricate procedures to measure pathogen aggressiveness. I was wondering about the method of extracting DNA from single pustules. Was DNA extracted from one single pustule on a leaf or were the spores from single pustule increased to sufficient amounts before extraction? If it's the former, I wonder about the non-specific binding of the primers to plant DNA during genotyping.

In the results sections, the authors presented the increase in frequencies of new genotypes within a pathotype. Again, I would view this as a result of evolution of effectors within a race in response to the resistance genes present in the field. This usually happens in rusts as they can have multiple ways to generate genetic variability. In the discussion, I think it would be better to mention less of the results. The authors also mentioned that quantitative resistance leads to more aggressive isolates, which is in contrast with what we observe with other rusts. Could the authors expound more about the potential mechanism behind this? I believe qualitative resistance imposes a stronger selection pressure because it induces hypersensitive response or 'complete resistance' which is a very effective immunity reaction, thus requiring the pathogen to evolve in order to survive. Also, I disagree with the new term 'pathogenotype' because it would create a confusion with the existing meanings of pathotype and genotype. I think 'lineage' would be enough to describe genotypes within a pathotype.

I like the outlook of using genomewide markers to characterize leaf rust populations in the future. With the availability of a P. triticina reference genome, several isolates can be genotyped and association studies can be conducted to map genes responsible for the aggressiveness phenotypes, especially that the authors have a very good system in place for phenotyping.

Reviewed by anonymous reviewer 2, 14 November 2022

This is a very interesting manuscript about genotype dynamics and characterisation within two pathotypes of the wheat rust P. triticina. The authors have mined a rich, decades-long survey collection of P. triticina isolates that allows them to explore dynamics of pathotypes and genotypes within pathotypes over time. They have identified two major pathotypes for exploration of genetic and phenotypic variation. They identified a pair of genotypes that decreased/increased in frequency recently, for both pathotypes, and measured three components of aggressiveness for the losing and the winning genotypes to test the hypothesis that the winning genotype would show higher aggressiveness pathotype (i.e., more efficient at infecting, more rapid at sporulating and producing more abundant spores) on common wheat varieties they are able to infect.

This may be the case for one out of two of the pathotypes, though I have some reservations about the statistical analyses and need to be reassured that these were carried out appropriately. If this proves to be so, the authors have demonstrated a case of evolution of enhanced in natural populations of P. triticina.

This is interesting and useful. However, I have some issues with the context (as well as with the statistical analyses). The authors frame their questions around quantitative (as distinct from qualitative) resistance, and the relation between aggressiveness and quantitative resistance is unclear to me. If aggressiveness or its components are fitness traits for the rust then it is not so surprising that aggressiveness will increase, because more aggressive genotypes should out-infect and out-transmit their less aggressive counterparts. This should be the case on host substrates with or without or with more or less quantitative resistance so I do not understand the connection between natural selection for increased aggressiveness and quantitative resistance of the host. It would be helpful if the authors could clarify this relationship. I understand that the phenotypes the authors measured as components of aggressiveness are expressions of, at the same time, the pathogen's ability to exploit the host and the host's degree of defence. However, one or both of these may vary, and the authors need to explain this and how they decide whether they are looking at host traits

(quantitative resistance), pathogen traits (aggressiveness) or the interactions of the two. That they tested all pathogen isolates on more than one host may allow them to detect when a particular phenotype varies among different pathogen genotypes, between or among host cultivars or in interaction, but I could not find formal tests for the interactions and perhaps the design did not allow comparisons of the host cultivars in all cases (but the statistical analyses are presented too superficially for the reader to really tell).

And for another smaller issue, it is suggested that "aggressiveness" implies "causing more damage to the host", i.e., more aggressive isolates will impact their hosts more. Of course it makes sense that higher infection efficiency, shorter latency period and higher spore production per lesion will cause more host damage, but this is always implicit and never explicitly explained or justified. I think the authors need to qualify this, either by explaining that these traits DO cause more host damage and give us the evidence or demonstration that biomass or yield or seed quality or whatever is decreased more by more aggressive isolates, or by stating that this is an underlying implicit assumption that has not yet been demonstrated.

I provide a number of detailed comments below, in order of their appearance in the manuscript. Some are just details of wording. Some are more substantial.

Abstract:

ln 18: "... with a particular combination of"

ln 20-21: this is rather vague. What aspect "could not be explained by..."? Do you mean that, given the R genes in the landscape, you would NOT have expected these pathotypes to decline? Were these pathotypes MORE or LESS common than you would expect from the r-profiles of the cultivated varieties? Please be specific and give as much information as possible.

Ln 25: "the more recent genotype was more aggressive than the older one."

Ln 28-29: your description of the "neutral" cultivar is not really adequate. If you need to put quotation marks this means that there is a problem with terminology you have not solved. Please try to explain this better. What do you mean by "no selection effect ..."?

Ln 29: For pathotype 106 314 0, the most recent genotype had a shorter latency period.

Ln 33 – 36: "A gain in aggressiveness allowed the maintenance of a declining pathotype, and even further expansion of that pathotype, in the pathogen population" What does this mean? If the pathotype is expanding how is it also declining?

"providing evidence that virulence alone is not sufficient, aggressiveness also being required for the adaptation of a pathogen to a changing varietal landscape.

Sorry but if "adaptation" means "evolution by natural selection" this makes no sense. What do you mean by "adaptation to a a changing varietal landscape"? Perhaps you mean that "Adaptation to a changing varietal landscape will not only affect/modify virulence but will also lead to changes in aggressiveness"?

Is this what you mean?

Introducton

Ln 51-54: You define "aggressiveness" in terms of pathogen damage to the host.

Ln 60: You list the characters used to assess "aggressiveness". Do these estimate damage to the host? Can you justify this?

Ln 63: "Infection efficiency is calculated as the proportion of spores that cause a new"

Ln 69: "The latency period is the length of time between inoculation and first sporulation

Ln 83-86: You state that sporulation capacity is dependent on the latency period but This does not automatically follow. Of course, interactions with longer latency periods will start to sporulate later. However, their sporulation capacity AFTER they have begun to sporulate may be independent of LP.

Ln 93: What does this "essential" mean?

Ln 101-106: I have several issues with this paragraph:

You are not describing ”population dynamics” here. I think you are describing ”which genotypes or pathotypes dominate the pathogen population” or ”pathogen population composition”.

Why do you expect quantitative resistance to select for increased aggressiveness? You tell us in Line 47 that qualitative resistance leads to an incompatible reaction, i.e., no infection. Hence it is pretty clear why qualitative resistance should select for the emergence of the corresponding virulence types. However, it is not clear what phenotypic effect quantitative resistance genes that you mention here have on the pathogen. Without this being clear (do quantitative resistance genes reduce infection efficiency or sporulation capacity or increase latency period? Is that why you expect them to exert selection on these traits?) we do not understand the link between quantitative resistance and your measures of aggressiveness.

You state that evolution of increased aggressiveness has been observed (but you do not tell us if this was in a context of quantitative resistance so the connection here is not clear at all.

Indeed, there may be selection for increased aggressiveness, IF aggressiveness increases reproductive success or fitness of the parasites. But that can happen in presence or in absence of quantitative resistance so it is not clear what these observations bring to the question about quantitative resistance.

Ln 119: “variation in…” But what does this last sentence refer to? Variation over time for what time scale? What is the time scale of “the complete life history of a pathotype”? What is the “life history of a pathotype”?

Ln 123: Can you be more precise here? Perhaps ”to determine whether information on aggressiveness allows us to predict changes in pathotype frequency” or something like that ??

Ln 124: again “the life history of what”?

Ln 125-126: Why are these pathotypes ”good case studies?” Can you justify this statement?

Ln 127-128: We first characterized isolates of these two pathotypes using microsatellite markers, to quantify their genotypic diversity”.

Ln 131: I think you do not need to give the pathotype identification twice in the same paragraph.

Ln 131-132: please replace “when relevant” by “for pathotype 106 314 0 only, (ii)…”

Methods:

Ln 138: “were” in place of “have been”

Ln 143-144: How was a ”single pustule isolate” obtained from a bulk harvest? Do you mean ”single-spore”? Or was one pustule per leaf sampled at the time of collection before the urediniospores from the leaf were bulked?

Ln 147-148: Perhaps here you could already tell us the years spanned by these samples and perhaps explain why you chose this short period for 166 317 0 versus the longer period for 106 314 0.

It appears that you assume that the bulk sample from the leaf shares the pathotype of the sampled pustule from that leaf. Is this assumption reasonable? Did you ever find more than one pathotype per leaf?

Ln 161-164: Did you genotype “one isolate” of the 44 or “one isolate per pathotype” of the 44?

You use the terms “pustule” and “uredinium”. Are these equivalent? If that I the case please choose one and use it throughout.

Ln 215-218: “Three components of aggressiveness, latency period, infection efficiency and sporulation capacity, were assessed for …”

Ln 219: Is it possible to tell us something about Apache’s resistance profile?

Ln 227-229: “In series 1 and 2, we tested whether the two genotypes of each pathotype differed in aggressiveness on …”

Ln 231-236: “Series 3, 4 and 5 tested the difference in aggressiveness between the two genotypes 106 314 0-G1 and 106 314 0-G2 on some or all of the wheat cultivars; Aubusson, Premio, Michigan Amber, Sankara, Expert and Bermude — all of which, except Michigan Amber, were among the 35 most frequently grown cultivars in the French landscape during the 2006-2016 period.

Ln 239-240: This statement is not consistent with Table S3 where I find

Isolate	Pathotype	Genotype	Series	Cultivar sampled	Cultivars tested

BT12M119	106 314 0	106 314 0-G1	1+4	Apache	Aubusson, Premio, Michigan Amber
BT12M033	106 314 0	106 314 0-G2	1+4	Apache	Aubusson, Premio, Michigan Amber

The table suggests that either series 1 or series 4 tested on Aubusson and Premio. However, these two strains should also have been tested on Apache, no?

Ln 242-243: Here or somewhere (perhaps in a table?) you should state on what dates these replicated and series were carried out.

Ln 252: "under binocular magnifier" should be "under a dissecting microscope".

Statistical analyses:

Your presentation of the statistical analyses are not really sufficient though this problem is much worse in the results. I cannot tell how your tests were performed and how you dealt with the hierarchical nature of your data (several observations per isolate, several isolates per genotype) for the non-parametric analyses.

All statistics, be they parametric or non-parametric, assume that each datum has the same degree of independence from all other data. This is not the case in your design and must, therefore, be analysed with a hierarchical model. Alternatively, you need to take the mean or median and analyse that. You certainly MAY NOT consider all data as though they are independent.

You appear to not test the interaction between cultivar and genotype. Why?

For series 3, 4 and 5 maybe you can test the three cultivars common to all (Aubusson, Premio, Michigan Amber) using Model 1. Might this give additional information?

Ln 287-288: Why do you write "to analyze the effect of genotype on the aggressiveness components." You also test the cultivar effect in some cases.

Results

Ln 295-296: Your description of the dynamics is not complete. Either just refer to the Figure, i.e., "The dynamics of the two pathotypes 106 314 0 and 106 314 0 in the French P. triticina population are shown on Figure 3." Or describe more completely, i.e., "The frequency of pathotype 106 314 0 in the French P. triticina population increased from 30% in 2006 to 51% in 2009 and decreased back to about 30% in 2011 (Figure 3). After a plateau at 30-33% from 2011 to 2014, the frequency of this pathotype decreased strongly, to less than 1% in 2018.

Ln 299: This should be "Figure 4".

Ln 301-307: I think this can be summarized as follows: "Genotype 106 314 0-G2 was the rarer of the two until 2012 after which it replaced genotype 106 314 0-G1. (Figure 4).

Ln 307-310: Since you told us that the cumulative frequency of G1 and G2 varied between 40 and 65% we already know that there were other genotypes that represented the remaining 35-60%. I think you can delete this.

Ln 313: "Its dynamics … showed two peaks …" Do you tell us how many samples were examined each year to generate these curves? How much confidence can we have in the first peak? Maybe it is just a sampling blip due to small sample sizes per year. I think here you also over-describe your results. Just say that the dynamics are shown in the figure. That is really enough.

Please trim and reduce the paragraph on lns 312-324.

I suggest: "Pathotype 166 317 0 was first detected in 2007 at a very low frequency (less than 2%; Figure 3). Two genotypes of this pathotype, differing by only one of the 19 SSR loci used (RB8; Table 3), were observed from 2013-2016 period, during which the initially dominant 166 317 0-G1 genotype was almost replaced by the 166 317 0-G2 genotype (Figure 5). Over the four-year period other genotypes accounted for 30% to 46% of this pathotype."

Ln 326-337: We need to see the analyses to be able to judge the reliability of these results. There is a problem of experimental design if you carry out a non-parametric analysis, I think, because I do not understand

how your degrees of freedom are calculated for the nested analysis. You must perform a nested analysis or correct for non-independence of data somehow. I think you have several observations per isolate and several isolated per genotype so this is clearly a hierarchical structure that must be analysed appropriately. Without seeing the calculations of the statistics with their dfs the reader cannot know that you have analysed your data correctly.

Without seeing your ANOVA tables and understanding how you dealt with data non-independence I cannot judge whether you found differences in aggressiveness or not.

Discussion:

The discussion is very long. Also it sometimes goes beyond where the data comfortable allow. The authors should soberly look at what they really have evidence for and stick with that.

I think there may be some over-interpretation of the results.

For pathotype 106 314 0 you find very little evidence for an increase in aggressiveness. Nonetheless you interpret the reduction in rate of decline in frequency of the pathotype between 2011 and 2014 as being due to a new more aggressive genotype arising. First, the genotype that increased in frequency was not much more aggressive. Second, it is not clear to me whether you genotyped all the isolates that are featured in Figure 3. Can you tell us the genotype frequencies of the two strains on that curve? If you have those data please plot them. You can make pie diagrams for each point to show the genotype frequencies for each observation. Without this the arguments are not that convincing. I really need more information to connect what I see on Figs 4 and 5 with the curves on Fig 3.

Ln 468: What is “mid-term”?

Ln 478-479: Do we really know that the change in frequency of pathotypes is driven by changes in resistance gene frequencies? Do we know what resistance genes are in the landscape? If we do, we should be able to predict which pathotypes will increase or decrease. Can we do that?

Ln 551: You qualify your G1 genotypes as “oldest” but you do not know their history, I think. Similarly the G2 may not be younger, necessarily. Perhaps these genotypes emerged in the past and happened to be at low frequency at the beginning of your sampling period. For 166 317 0 you cannot really know which one is older though they appear to be closely related.

Ln 556: Differing at several loci does not mean that 106 314 0 has “more genetic diversity”. Here what you can say is that the two genotypes are more differentiated. You would need to compare the number of different genotypes in similarly sized samples to compare genetic diversity.

Ln 557-559: The relationship between microsatellite, presumably neutral mutations and phenotypically relevant mutations is not very straightforward. That the two genotypes of 106 314 0 are more differentiated for these neutral markers does not necessarily mean that they are more differentiated for functional mutations. In fact, you found LESS phenotypic variation for your aggressiveness traits.

603-604: What does “Conversely, identical genotypes may differ in one or several virulences.” mean?

I am not sure that “pathogenotype” will be a very useful term.