nature portfolio

Peer Review File

Population genomics unravels a lag phase during the global fall armyworm invasion

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

In this study, the authors use genomic data to infer the presence of a lag phase – or its lack thereof – during the recent invasion history of the fall armyworm (FAW). I have read the MS with much attention and found it very interesting, particularly considering that the usage of genomic data to understand the demographic history of invasive species is still limited. Although I found the study very interesting, there are some important aspects that I think should be considered before publication:

1) I am not entirely convinced about how testing alternative classic models of divergence (SI, IM, AM, and SC) can help to infer the presence/absence of a lag phase, particularly considering that the different demographic parameters were not actually estimated here. Statistical support for a model of strict isolation (SI) could also potentially support the presence of a lag phase: e.g., native and invasive populations diverged without gene flow but it took a long time for invading populations to spread after an initial bottleneck following colonization of the invaded range by a limited number of propagules. Estimating the time elapsed between divergence and the demographic expansion could help to determine the length of the lag phase. There are some obvious limitations on parameter estimation (i.e., uncertainties around mutation rates and generation times), but at least we could get an idea about the presence and, more importantly, the length of the lag phase.

2) Linked to the previous point, I would also encourage the authors to test alternative demographic scenarios considering population bottlenecks and expansions. The time elapsed between the expected bottlenecks and demographic expansions of introduced populations would be informative of the length of the lag phase (not only whether it is present or not). See the study by Ortego et al. (Molecular Ecology, 30: 4189-4203), one of the few published papers using genomic data to infer lag phases from coalescent-based demographic model testing. This study should be probably cited in introduction section (e.g., line 63) and considered for discussing your results in the context of previous research.

3) L236, it is not clear to me why only the population from Benin was analyzed here. The population from India also has a relatively large sample size (n = 14, L221) and a similar invasion history (L103). Stronger conclusions could be reached if you analyze the data across these two population replicates.

Minor comments:

4) L75-78, if I understood well, lineages mtA and mtB did not independently and repeatedly admix in different parts of the invaded range, such admixture happened only once before the invasion of the different regions in Africa and Eurasia (?). Please, make this important aspect clear.

5) L84-87 (linked to the previous point): I do not follow this argument. Do you mean that admixture took place multiple times (and independently) in the different parts of the invaded range? What do

you mean with “repeatedly observed patterns”? The fact that two studies on the same system have reached the same conclusion does not mean that the same process has taken place repeatedly or independently. Sorry, I admit that I have not read the previous studies on the invasion genomics of FAW, but I guess that any reader should be able to understand these basic aspects without the need to go through previously published papers.

6) L172-173: Note that purging of deleterious alleles after a severe bottleneck following introduction is expected to alleviate the negative consequences of inbreeding in recently founded populations.

7) L177-181: In the case of the FAW, the relatively high level of genetic diversity in invasive populations compared to native ones could be simply the outcome of genetic admixture between the two mtDNA lineages. In other words, probably, genetic diversity did not increase during the lag phase, but as a consequence of admixture before the spread.

8) L184-185: I agree that it is highly likely that many of the alleles only present in invasive populations have not been sampled in the native range. I did not think too much about this, but I wonder if low Tajima´s D in invasive population specific SNVs is simply due to the fact that they are very rare alleles, which could also make them very difficult to detect among your (necessarily limited) sampled native populations.

Reviewer #2 (Remarks to the Author):

Summary

Durand et al. use population genomic summary statistics to show that invasive fall armyworm (FAW) populations were established before they were first reported in 2016. This approach is interesting and novel – I see it being applicable to many other study systems. I found the results based on the age of derived invasive-specific alleles to be compelling. That being said, the results based on genome-wide summary statistics provided very little biologically meaningful information because they are jointly influenced by admixture and demography. Overall, the results support the conclusion that the FAW was present in the invasive range before its discovery, though this finding is already widely accepted. The inferences made here are somewhat limited because they can be explained by ‘inherent lag’ – a lag phase that is simply the result of low population sizes in the early phase of exponential growth, which is to be expected in any biological invasion. I recommend a more critical discussion of the results and a more nuanced discussion of how population genomics can be used to study lag phases. I enjoyed reading this paper and I thank the authors for reporting their study so clearly and concisely.

General comments

1: Both demography and admixture influence the summary statistics presented in figures 2 and 3

The consensus based on previous work is that invasive FAW populations are admixed and result from multiple invasion events (for example, it is known that both corn and rice strain mitochondrial haplotypes segregate in invasive populations). The demographic history of invasive populations is therefore complex. The authors interpret patterns of heterozygosity and Tajima's D as evidence of population growth in the invasive range, and argue that the observed signal of population growth would take a substantial amount of time to appear. The complex demographic history in this system makes these statistics difficult to interpret on their own. Although I agree that the observed statistics are difficult to explain if the invasive populations established shortly before 2016, the authors should acknowledge that the confounding effects of demography and admixture mean that there are other possible explanations for the data.

First, the source populations are not at demographic equilibrium; although Tajima's D is negative in the invasive range, the statistic is significantly more negative in all native populations. Given the implicit assumption that the invasive population is ultimately a sub-sample of native populations, this should affect how the authors interpret and discuss Tajima's D in the invasive range.

Second, Tajima's D is affected not only by the timing of a population bottleneck but also by its magnitude. Can the authors rule out the possibility of a large, recently established invasive population based on these statistics?

Third, admixture affects both statistics. The invasive population is admixed and may have experienced multiple bouts of gene flow from different source populations, which likely explains why the reduction in heterozygosity is only marginal in invasive populations. Admixture may have the effect of generating an excess of rare variants in the invasive range, thereby decreasing Tajima's D.

The points above should be discussed, and the authors should make it clearer that all their analyses implicitly assume comprehensive sampling of native populations.

2: The results can be explained by 'inherent lag', which is a feature of any biological invasion

It seems likely that invasive FAW populations underwent a prolonged lag phase driven by complex invasion dynamics and eco-evolutionary feedbacks. The results presented here, however, simply show that the invasive populations established before they were discovered, which is unsurprising. There will always a difference between the time an invasive species arrives and the time at which it reaches the critical population size at which it is detectable. The very nature of exponential growth in the early phase from a small founding population means that this period may be quite long; an 'inherent' lag phase. In general, researchers are more interested in extended lag phases – decades or more in some cases – in which an established population had persisted at a small, undetectable size because the ecological or evolutionary conditions for exponential population growth had not yet been met. Testing this requires showing that the period between arrival and detection could not be explained by exponential population growth alone.

In this study, the authors show that (1) invasive FAW populations are probably growing, (3) there has

been sufficient time for invasive-specific alleles to arise, and (3) that there is a genomic signature of a cessation of gene flow from source populations (assuming comprehensive sampling of the native range). These results are entirely consistent with immediate exponential population growth from a small founding population. This is unsurprising and doesn't shed light on why FAW populations are so widely distributed and genetically diverse. Ideally the authors would estimate the amount of time required for the observed invasive-specific alleles to arise, or the minimum amount of time required for evidence of reduced gene flow to occur. I can see that this is not straightforward in this study, as knowledge of the FAW invasion history and the relationship between source and invasive populations remains obscure. I encourage the authors to discuss how their approach might be applied in other study systems, and how the approach can be used to inform management and biosecurity failures.

Minor comments

L84-89: Why do the authors assume that hybridization did not occur in the native range, such that the invasive population was already admixed upon arrival?

L86: "admixtures" should be "admixture"

L114-115: I'm not sure what "… due to the increase after a genetic bottleneck" means here. Increase in what? A genetic bottleneck is a reduction in population size followed by an increase in population size. Heterozygosity is reduced in the invasive populations, consistent with the loss of genetic diversity following a bottleneck. Evidently there has not been sufficient time for heterozygosity to regenerate.

L137-138: Explain "IM," "SC" etc. here

Reviewer #3 (Remarks to the Author):

The manuscript "Population genomics unravels an undocumented lag phase during the global fall armyworm invasion" attempts to elucidate the presence of a lag phase during the global invasion of the fall armyworm. While I agree that determining the specifics of a species lag phase are important in being able to gauge its aptitude for further spread (and thus, better contain the potential spread), this work appears to be mostly superficial and I'm not sure the results necessarily support the authors' assertions. As it stands now, the study requires significant expansion and clarification if it is to be considered for publication.

Comment 1: L114-L116 – "The slight difference in heterozygosity between native and invasive populations may result from a moderate decline after an introduction or due to the increase after a genetic bottleneck. In the former case, Tajima's D calculated from invasive populations is expected to have a positive sign." I'm not sure this is true, and this statement seems to be your sole basis for your assertion that a lag phase occurred. A lag phase likely did occur, but I'm not sure how a

negative Tajima's D conclusively supports that. Looking at Figure 3, it seems that each native population has a lower Tajima's D, so if the colonizing population was a decent enough size (and originated from a native population with a highly negative Tajima's D) could this not also explain the negative Tajima's D within the invasive populations (among many other possible explanations). I think you need some simulations here showing what you claim is true.

Comment 2: Let's assume that a negative Tajima's D for an invasive population is truly indicative that a lag phase has occurred (or that the simulations you do above show this to be likely). Wouldn't it make sense then to try to determine the length of this lag phase? You could use pop gen simulation software to estimate the generations needed to decrease Tajima's D to the mark it is now (if you're assuming the initial colonizing population is >0). The second sentence of your abstract makes it seem like this might be a question you attempt to answer, so I was a little disappointed not to see this already.

Besides the above, several details regarding the study require further clarification:
L223: Readers should not have to parse a separate paper to figure out the methods used for the paper they're currently reading.
L224-230: What is the justification for 100kb windows? Also, it would be interesting to see a plot along each autosome (maybe some interesting patterns would emerge).
L244-L246: Why not perform an actual linkage analysis to determine linkage values for each chromosome rather than this arbitrary selection of snps? This would provide you with an appropriate window size to use for your stats calculations as well.
Figures 2, 3, 4: Why don't you use the same populations for each analysis (eg, China, French Guiana, Guadeloupe in Fig 2 but not Fig 3; China's not in Figure 4 but it's invasive)? Also, for Figure 2, it seems like there are two individuals lacking heterozygosity (India samples)? Finally, the populations of Brazil, Uganda, and Malawi are listed in the methods but they aren't present in any figure?

Finally, it seems this paper was originally prepared with the methods section placed before the Results and Discussion and not properly adjusted once the Methods were moved after these sections, which leads to a not-so-clear final product. I think a lot of the confusion I encountered could be remedied by placing select details from the Methods section into the Results and Discussion sections. For instance, the different models used for the diffusion analysis are referred to using acronyms in the Results section (where the reader first encounters them), so it is really not clear what the different models are until one gets to the Methods section.

Reviewer #4 (Remarks to the Author):

General comment

In this manuscript, Durand et al. propose to use genomics approaches (instead of data from historical reports) to better identify "lag phases" during biological invasions. For this purpose, they re-analyze a consequent whole genome dataset of 177 Spodoptera frugiperda samples from both

native and invasive populations, recently published (Yainna et al. 2022, Scientific Reports). I found the article to be generally well written and to address an interesting question on the dynamics of invasions. The analyses include estimations of genetic diversity, tests for deviation from equilibrium, and demographic inference. I provide several suggestions to clarify between demographics vs. genomics, as well as on methods and presentation of the results that I hope will be helpful for improving the manuscript.

My major comment mainly concerns demographic inference. Based on figure 1, I expected the models to include (1) the ancestral admixture (figure 1C), which estimated date is important to address the research question, and (2) the comparison between a reduction of Ne (founder effect) during a given time period followed by an exponential increase in Ne (population expansion; allowing a more ancient introduction date) (figure 1A) versus immediate increase in Ne without founder effect (figure 1B; imposed to known introduction date). I found confusing the choice of samples for the analyses, e.g., including sfR for Ho and Tajima's tests; only Benin (not India) for invasive populations and only mtA (not mtB; but I did not find the information in the methods) for native populations, while excluding Mexico. Figures and tables captions also need revision, e.g., I guess the 'sfR' samples in figure 2 and figure 3 correspond to the rice strain; what do the different panels correspond to in figure 3; expand scenario codes in table 1.

Specific comments

Keywords: add relevant keywords about wgs data

L.43: which component of diversity?

L.47-49: I agree that time for adaptive evolution has been proposed to explain the lag times between introduction and spread. But one common explanation is a demographic issue (negative density dependence).

L.55-57: In addition to this consideration, historical records are largely incomplete for most invasive species.

L.58-61: Clearly explain the link in between here. For example, genomic data combined with demographic modeling help deciphering the geographic origin (spatial sampling) or whether the current population is a daughter population of early-detected propagules (temporal sampling); changes in effective population size (Ne) inferred from genomic data can reveal a reduction in Ne followed by an increase in Ne, which can reflect the impact of demographic changes (founder event, population expansion/migration, respectively); etc. This can be clarified providing example studies.

L.62-63: I do have a question regarding "the socio-ecological importance of identifying lag phases in invasive species (L.61-62)", because "it generally corresponds to the last opportunity to eradicate an alien pest species (L.13-14)". Considering a study that 1) manages to collect samples just a few

generations after an introduction; 2) performs sequencing and analysis of genomic data to determine whether (or not) and when such an initial “lag phase" has occurred; 3) reveals changes in Ne over time that could be indicative of a lag phase (see my comment on L.58-61); what are the potential implications in terms of population management? Do the studied populations still have the same demographic status? For example the present study: 6 years after collection so potentially 60 generations based L.137.

L.83: Delete the space after the opening parenthesis.

L.75-89: There are a couple of studies described here, the results of which are very important because they correspond to the knowledge upon which the hypotheses of the study are based. However I don’t find that Figure 1 is sufficiently explicit. I suggest the authors to show a multi-panel figure summarizing (1) the sampling, (2) the results of previous studies, and (3) the competing scenarios of Figure 1A and 1B, which should be the ones tested in demographic inference (figure S1).

L.85-89: Reformulate.

All invasive populations show a common pattern of admixture between native mtA and mtB strains, suggesting a common origin [...]. If these repeatedly observed patterns are true, it is tempting to hypothesize that the introduction of FAW in Benin is more ancient than currently thought. The homogenization of genome sequences after the admixture event is unlikely to have emerged over the course of just a few generations (~10 generations). Therefore, a founding admixture in Benin (or India) is expected to have occurred (and thus the introduction of FAW) before the detection date of the species (2016) because of the existence of a lag phase where the species remained undetected (i.e., too low population size). Population genetic variation can be used to test for the impact of a bottleneck during introduction (but not necessarily true due to the admixture event) and of a subsequent recent expansion. Furthermore, we should observe incongruence between the time of the admixture event and the time of detection.

Following this hypothesis, the invasive population in Benin (or India) initiated all other introductions worldwide? I want to stress that the discussion L.184-187 on the fact that the ancestral admixture might have occurred in an unknown location within the native range, from which populations were independently introduced to Africa and Asia, has to remain in future versions of the manuscript.

L.102: repetition of ‘statistical’

L.103: Are populations in Benin and India genetically differentiated? i.e., is it possible to differentiate these scenarios: (1) founding admixture between mtA and mtB in Benin then spread to other invaded areas, (2) founding admixture in India then spread to other invaded areas, (3) ancestral admixture (ghost population) then independent introductions in Benin and India?

L.113: I suggest to provide the distribution of individual observed heterozygosity by population (and provide this figure 2 in supplementary material). This will help follow the text on comparisons

between native/invasive populations.

L.114: Also in figure 2, there is one sample with higher heterozygosity. Is this sample admixed with another population?

L.115: “moderate decline or due to an increase after bottleneck”. Did the authors test for the impact of a founder effect and subsequent increase in effective population size?

L.120: Tajima’s D average estimates are negative for invasive populations in Benin and India, suggesting these populations are expanding. Are values of Tajima’s D similar in native and invasive populations?

L.125: Increase font size for sample IDs. Are these two individuals from India (with lower pop-specific SNVs) also those with almost completely homozygous genomes in figure 2? What is the coverage for these two samples?

L.135-136: The authors assume that a cessation of gene flow would be "indicative" of the existence of a lag phase. Can processes other than cessation gene flow leave the same signature in the SFS? I agree that 10 generations can be a little short to obtain genetic differentiation between native and invasive populations from the accumulation of independent mutations/genetic drift due to geographic isolation, especially if the initial effective population size was large. From a highly diverse admixed population, could a founder effect of medium intensity result in the observed patterns of heterozygosity?

L.138-143: Provide the full name of scenarios at first use, especially because of the format of the article (methods at the end). More generally, the authors may consider having a sentence summarizing the methods at the beginning of each paragraph.

L.140: Table 1. Why only testing for Benin and not India?

L.147-148: see my comment on L.120.

L.169: "Lag phases explain the genetic paradox of invasion": ?

L.178-180: I find this discussion here about bottlenecks a bit confusing given that previous studies have demonstrated an ancestral admixture common to all invasive populations and that the present study, in agreement, supports very slight reduction in heterozygosity.

L.180-181: What was is the inferred duration of the lag phase? The authors do not provide or discuss population parameter estimates from demographic inference (posterior distributions for best scenario). Why?

L.188: mtA only in the native pools of demographic models? Justify. Could the results be impacted by an unmodelled demographic event (e.g., ancestral admixture, expansion)?

L.228: population structure

L.229: ‘average Tajima’s D over windows’

L.256-261: Provide the prior distribution for population parameters.

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

In this study, the authors use genomic data to infer the presence of a lag phase – or its lack thereof – during the recent invasion history of the fall armyworm (FAW). I have read the MS with much attention and found it very interesting, particularly considering that the usage of genomic data to understand the demographic history of invasive species is still limited. Although I found the study very interesting, there are some important aspects that I think should be considered before publication:
R1: Thank you for your comments. We agree with all your comments from you. We believe that the manuscript is significantly improved by answering your comments.

1) I am not entirely convinced about how testing alternative classic models of divergence (SI, IM, AM, and SC) can help to infer the presence /absence of a lag phase, particularly considering that the different demographic parameters were not actually estimated here. Statistical support for a model of strict isolation (SI) could also potentially support the presence of a lag phase: e.g., native and invasive populations diverged without gene flow but it took a long time for invading populations to spread after an initial bottleneck following colonization of the invaded range by a limited number of propagules. Estimating the time elapsed between divergence and the demographic expansion could help to determine the length of the lag phase. There are some obvious limitations on parameter estimation (i.e., uncertainties around mutation rates and generation times), but at least we could get an idea about the presence and, more importantly, the length of the lag phase.
R1-1: Thank you for your comment. We completely agree with your point, which has also been noted by other reviewers (R2-2, R3-2a, R4-1, and R4-19).

We acknowledge that estimating the lag phase can be a more robust approach for testing the lag phase than model selection because we can circumvent the need to assume that a lag phase will necessarily yield detectable time without gene flow.

Hence, we performed parameter estimations to determine the duration of the lag phase. We found that the AM_bottl model was selected over other demographic models. Subsequently, we estimated the time of introduction (*Ts*) from this model.

Although the 95% bootstrapping confidence intervals of *Ts* was relatively wide, obviously *Ts* predates the expected time without gene flow (one year) supporting the existence of the lag phase.

We inserted the result in the manuscript (Fig. 5, Fig. S8, L167-L168, L180 – L188, L199 – L200, and L335 – L337).

2) Linked to the previous point, I would also encourage the authors to test alternative demographic scenarios considering population bottlenecks and expansions. The time elapsed between the expected bottlenecks and demographic expansions of introduced populations would be informative of the length of the lag phase (not only whether it is present or not). See the study by Ortego et al. (Molecular Ecology, 30: 4189-4203), one of the few published papers using genomic data to infer lag phases from coalescent-based demographic model testing. This study should be probably cited in introduction section (e.g., line 63) and considered for discussing your results in the context of previous research.

R1-2: Thank you for your response. We fully acknowledge the importance of incorporating a realistic demographic history of invasion, as highlighted not only by your comment but also by another reviewer (R4-1).

In our efforts to address this aspect, we extensively explored models that could capture the demographic history of invasion, particularly scenarios involving bottleneck followed by expansion. However, we encountered challenges in identifying a suitable model that converged without issues while avoiding excessive parameterization.

Ultimately, after careful consideration, we selected the AM_bottle model, which involves a split between ancestral native and invasive populations, followed by the cessation of gene flow and a bottleneck event. We also evaluated the AM_bottleGrowth model, which incorporates population growth after the bottleneck. We observed that AM_bottle had always lower AIC than AM_bottleGrowth (please see Table1 below in the attached file -Additional_info.pdf), implying that AM_bottle outperformed AM_bottleGrowth in terms of the goodness of fit and the complexity of models. Furthermore, AM_bottleGrowth had always lower likelihood than AM_bottle, showing that optimal parameters of population expansion are not reliably estimated in AM_bottleGrowth.

We speculate that the inability of AM_bottleGrowth to accurately estimate optimal parameters for population expansion may be attributed to the recency of population expansion in the invasive populations relative to the number of samples analyzed.

Given these considerations, we decided to report the results obtained from the AM_bottle model (Fig. S6, Fig. S7, L171-L179, L320-L321, and L323-L327) with the citation of the suggested paper (L176-L177).

3) L236, it is not clear to me why only the population from Benin was analyzed here. The population from India also has a relatively large sample size (n = 14, L221) and a similar invasion history (L103). Stronger conclusions could be reached if you analyze the data across these two population replicates.
R1-3: Thank you for your comment. This point was mentioned by another reviewer (R4-2 and R4-21).

We replicated all analyses with the Indian population, and we observed comparable patterns with the population from Benin. We inserted this result (Fig.5, Table S1, Fig. S7, Fig. S8, L169-L170, and L183-L186).

Minor comments:

4) L75-78, if I understood well, lineages mtA and mtB did not independently and repeatedly admix in different parts of the invaded range, such admixture happened only once before the invasion of the different regions in Africa and Eurasia (?). Please, make this important aspect clear.
R1-4: We acknowledge that the sentence can be confusing. We attempted to write it clearly (L70-L73).

5) L84-87 (linked to the previous point): I do not follow this argument. Do you mean that admixture took place multiple times (and independently) in the different parts of the invaded range? What do you mean with “repeatedly observed patterns”? The fact that two studies on the same system have reached the same conclusion does not mean that the same process has taken place repeatedly or independently. Sorry, I admit that I have not read the previous studies on the invasion genomics of FAW, but I guess that any reader should be able to understand these basic aspects without the need to go through previously published papers.

R1-5: Thank you for pointing out this lack of clarity. We completely revised the sentence based on the suggestion from another reviewer (R4-11) (L77-L82).

6) L172-173: Note that purging of deleterious alleles after a severe bottleneck following introduction is expected to alleviate the negative consequences of inbreeding in recently founded populations.
R1-6: We agree with you that inbreeding depression should be reduced once purifying selection removes deleterious alleles. The probability of showing inbreeding depression is a function of the times required for purifying selection and for generating deleterious homozygous alleles. We revised a sentence such that inbreeding depression may occur before the completion of purifying selection (L219-L221).

7) L177-181: In the case of the FAW, the relatively high level of genetic diversity in invasive populations compared to native ones could be simply the outcome of genetic admixture between the two mtDNA lineages. In other words, probably, genetic diversity did not increase during the lag phase, but as a consequence of admixture before the spread.
R1-7: We absolutely agree with you that genetic admixture can also increase heterozygosity. This point was mentioned by other reviewers as well (R2-1c, R4-15, and R4-24).

We tested this possibility by calculating the proportion of mtA-specific or mtB-specific variations in each invasive sample. We observed that mtA-specific SNVs account for 8.63% - 11.8%, while mtB-specific SNVs contribute 0.902% - 1.31%. This result suggests that genetic admixture could increase heterozygosity by around, at most, 1%. We inserted this result (Fig. S3 and L116-L122).

8) L184-185: I agree that it is highly likely that many of the alleles only present in invasive populations have not been sampled in the native range. I did not think too much about this, but I wonder if low Tajima´s D in invasive population specific SNVs is simply due to the fact that they are very rare alleles, which could also make them very difficult to detect among your (necessarily limited) sampled native populations.
R1-8: Thank you for your comment. If the identified invasive population-specific SNVs originated from very rare alleles in native populations, it is challenging to elucidate how these rare alleles were introduced and subsequently maintained in invasive populations to such an extent that they constitute 5.19% to 6.76% of the total SNVs. We added this logic in the manuscript (L241-L247).

Reviewer #2 (Remarks to the Author):

Summary

Durand et al. use population genomic summary statistics to show that invasive fall armyworm (FAW) populations were established before they were first reported in 2016. This approach is interesting and novel – I see it being applicable to many other study systems. I found the results based on the age of derived invasive-specific alleles to be compelling. That being said, the results based on genome-wide summary statistics provided very little biologically meaningful information because they are jointly influenced by admixture and demography. Overall, the results support the conclusion that the FAW was present in the invasive range before its discovery, though this finding is already widely accepted. The inferences made here are somewhat limited because they can be explained by ‘inherent lag’ – a lag phase that is simply the result of low population sizes in the early phase of exponential growth, which is to be expected in any biological invasion. I recommend a more critical discussion of the results and a more nuanced discussion of how population genomics can be used to study lag phases. I enjoyed reading this paper and I thank the authors for reporting their study so clearly and concisely.

R2: Thank you very much for your comments. All your comments were relevant, and we did our best efforts to improve the manuscript according to your suggestions.

General comments

1: Both demography and admixture influence the summary statistics presented in figures 2 and 3

The consensus based on previous work is that invasive FAW populations are admixed and result from multiple invasion events (for example, it is known that both corn and rice strain mitochondrial haplotypes segregate in invasive populations). The demographic history of invasive populations is therefore complex. The authors interpret patterns of heterozygosity and Tajima's D as evidence of population growth in the invasive range, and argue that the observed signal of population growth would take a substantial amount of time to appear. The complex demographic history in this system makes these statistics difficult to interpret on their own. Although I agree that the observed statistics are difficult to explain if the invasive populations established shortly before 2016, the authors should acknowledge that the confounding effects of demography and admixture mean that there are other possible explanations for the data.

First, the source populations are not at demographic equilibrium; although Tajima's D is negative in the invasive range, the statistic is significantly more negative in all native populations. Given the implicit assumption that the invasive population is ultimately a sub-sample of native populations, this should affect how the authors interpret and discuss Tajima's D in the invasive range.
R2-1a: Certainly, the non-equilibrium status of the native population could significantly impact Tajima's D in the invasive population. This point was mentioned by other reviewers (R3-1 and R4-19).

To explore this possibility, we conducted coalescent simulations. Assuming an original population size of 10^6, we examined an extreme scenario where this population underwent a tenfold increase in growth 100 generations ago, followed by a bottleneck event ten generations ago. In this simulation, even under the unrealistic assumption of the simultaneous introduction of 10,000 unrelated FAW individuals, we observed that Tajima's D could not fall below zero. We included this new result (L144-L153, L300-L304, and Fig. S5).

Second, Tajima's D is affected not only by the timing of a population bottleneck but also by its magnitude. Can the authors rule out the possibility of a large, recently established invasive population based on these statistics?
R2-1b: You are absolutely right. This point was also mentioned by other reviewers (R3-1 and R4-19). We conducted simulations to test if a large number of introduced insects can generate a positive sign of Tajima's D. The simulation showed that the introduction of 10,000 unrelated individuals out of a total of 10^6 insects indeed led to a positive sign of Tajima's D. Since this number of introduced insects is unrealistically high, it is unlikely that the positive sign of Tajima's D was solely caused by the introduction of numerous insects. We included this new result in the manuscript (Fig. S4, L126-L135, and L292-L300).

Third, admixture affects both statistics. The invasive population is admixed and may have experienced multiple bouts of gene flow from different source populations, which likely explains why the reduction in heterozygosity is only marginal in invasive populations. Admixture may have the effect of generating an excess of rare variants in the invasive range, thereby decreasing Tajima's D.
R2-1c: We absolutely agree with you. This point was mentioned by other reviewers as well (R1-7, R4-15, and R4-24).

To investigate the potential impact of genetic admixture on heterozygosity in invasive populations, we calculated mt-A and mt-B specific variations for each invasive sample. Our findings reveal that mt-A and mt-B specific variations contribute to 8.63% - 11.8% and 0.902% - 1.31%, respectively. This result suggests that genetic admixture may have increased heterozygosity by approximately 1% at most. We inserted this result (L116-L122 and Fig. S2).

The points above should be discussed, and the authors should make it clearer that all their analyses implicitly assume comprehensive sampling of native populations.

2: The results can be explained by 'inherent lag', which is a feature of any biological invasion

It seems likely that invasive FAW populations underwent a prolonged lag phase driven by complex invasion dynamics and eco-evolutionary feedbacks. The results presented here, however, simply show that the invasive populations established before they were discovered, which is unsurprising. There will always a difference between the time an invasive species arrives and the time at which it reaches the critical population size at which it is detectable. The very nature of exponential growth in the early phase from a small founding population means that this period may be quite long; an 'inherent' lag phase. In general, researchers are more interested in extended lag phases – decades or more in some cases – in which an established population had persisted at a small, undetectable size because the ecological or evolutionary conditions for exponential population growth had not yet been met. Testing this requires showing that the period between arrival and detection could not be explained by exponential population growth alone.

In this study, the authors show that (1) invasive FAW populations are probably growing, (3) there has been sufficient time for invasive-specific alleles to arise, and (3) that there is a genomic signature of a cessation of gene flow from source populations (assuming comprehensive sampling of the native range). These results are entirely consistent with immediate exponential population growth from a small founding population. This is unsurprising and doesn't shed light on why FAW populations are so widely distributed and genetically diverse. Ideally the authors would estimate the amount of time required for the observed invasive-specific alleles to arise, or the minimum amount of time required for evidence of reduced gene flow to occur. I can see that this is not straightforward in this study, as knowledge of the FAW invasion history and the relationship between source and invasive populations remains obscure.
R2-2: Thank you for your comment. We agree with your point regarding the importance of estimating the time of introduction to determine whether a time gap between introduction and invasion can indeed be considered a scientifically meaningful lag phase. This point was also mentioned by other reviewers (R1-1, R3-2a, R4-1, and R4-19), further emphasizing its significance.

Simultaneously, it is important to acknowledge that numerous researchers still argue against the existence of a time gap in the case of fall armyworms. They posit that invasive fall armyworms rapidly disseminated across sub-Saharan Africa immediately upon reaching West Africa from the USA. Given these contrasting viewpoints, we believe it is meaningful to present findings related to the time gap between introduction and invasion.

To quantitatively estimate the lag phase, we used ∂a∂i through model selection and parameter estimation. Through the likelihood ratio test, we chose the AM_bottle model involving the split between ancestral native and invasive populations with gene flow followed by the cessation of gene flow and bottleneck. Then, we checked the time of cessation of gene flow (*Ts*) with the assumption that this time corresponds to the time of introduction. Although the calculated *Ts* had a wide 95% confidence interval, it was evident that *Ts* is far more ancient than the expectation without gene flow. This result clearly supports the presence of a lag phase.

We inserted this result in the revised manuscript (L167-L168, L180 – L188, L199 – L200, L335 – L337, Fig. 5, and Fig. S8).

We also tried to use AM_bottleGrowth, which includes exponential population growth after the bottleneck of introduced populations. However, we observed that AM_bottle outperformed AM_bottleGrowth in terms of the goodness of fit and the complexity of models (Please refer to Table 1 in the attached file provided to the reviewer). Thus, we decided not to include the result of AM_bottleGrowth.

I encourage the authors to discuss how their approach might be applied in other study systems, and how the approach can be used to inform management and biosecurity failures.
R2-3: Thank you for this excellent comment. We mentioned this issue at the end of the discussion (L9 – L10 and L270-L272).

Minor comments

L84-89: Why do the authors assume that hybridization did not occur in the native range, such that the invasive population was already admixed upon arrival?
R2-4: Thank you for this comment. Indeed, we cannot exclude the possibility that hybridization may have occurred in the native area. We adjusted the wording in the sentence (L73).

L86: “admixtures” should be “admixture”
R2-5: Thank you for pointing out this mistake. We removed the relevant sentence in response to the comment provided by other reviewers (R1-5 and R4-11).

L114-115: I’m not sure what “… due to the increase after a genetic bottleneck” means here. Increase in what? A genetic bottleneck is a reduction in population size followed by an increase in population size. Heterozygosity is reduced in the invasive populations, consistent with the loss of genetic diversity following a bottleneck. Evidently there has not been sufficient time for heterozygosity to regenerate.
R2-6: Thank you for your comment. We agree that the sentence was not written clearly. We revised the sentence (L123-L125).

L137-138: Explain “IM,” “SC” etc. here
R2-7: We added the description of the models (L170-L179).

Reviewer #3 (Remarks to the Author):

The manuscript “Population genomics unravels an undocumented lag phase during the global fall armyworm invasion” attempts to elucidate the presence of a lag phase during the global invasion of the fall armyworm. While I agree that determining the specifics of a species lag phase are important in being able to gauge its aptitude for further spread (and thus, better contain the potential spread), this work appears to be mostly superficial and I’m not sure the results necessarily support the authors’ assertions. As it stands now, the study requires significant expansion and clarification if it is to be considered for publication.
R3. Thank you for your review, and we sincerely appreciate it. We invested significant effort to improve the manuscript by answering your comments.

Comment 1: L114-L116 – “The slight difference in heterozygosity between native and invasive populations may result from a moderate decline after an introduction or due to the increase after a genetic bottleneck. In the former case, Tajima’s D calculated from invasive populations is expected to have a positive sign.” I’m not sure this is true, and this statement seems to be your sole basis for

your assertion that a lag phase occurred. A lag phase likely did occur, but I'm not sure how a negative Tajima's D conclusively supports that. Looking at Figure 3, it seems that each native population has a lower Tajima's D, so if the colonizing population was a decent enough size (and originated from a native population with a highly negative Tajima's D) could this not also explain the negative Tajima's D within the invasive populations (among many other possible explanations). I think you need some simulations here showing what you claim is true.
R3-1: Thank you very much for your comment. We completely agree with you, and this point was also mentioned by other reviewers (R2-1a, R2-1b, and R4-19).

We performed coalescent simulations with varying bottleneck strengths to test if very recent introductions could generate positive signs in Tajima's D. We also performed simulations to test if population growth in the native population could lead to negative signs in Tajima's D despite a bottleneck.

The results indicate that the introduction of 10,000 genetically unrelated individuals out of 10^6 insects is sufficient to produce positive signs in Tajima's D. Furthermore, in an extreme case where there was a 10-fold population expansion 100 generations ago, the negative sign in Tajima's D could not be generated even with the introduction of 10,000 or 1,000 individuals.

We inserted these new results (L126-L135, L144-L153, L292-L300, L300-L304, Fig. S4, and Fig. S5).

Comment 2: Let's assume that a negative Tajima's D for an invasive population is truly indicative that a lag phase has occurred (or that the simulations you do above show this to be likely). Wouldn't it make sense then to try to determine the length of this lag phase? You could use pop gen simulation software to estimate the generations needed to decrease Tajima's D to the mark it is now (if you're assuming the initial colonizing population is >0).

R3-2a: Thank you for your comment. We agree with your suggestion regarding the estimation of the time of the lag phase, and this point was also raised by other reviewers (R1-1, R2-2, R4-1, and R4-19).

While we acknowledge the potential utility of Approximate Bayesian Computation in estimating the time of the lag phase, we have concerns about relying solely on Tajima's D as a summary statistic for this purpose.

Instead, we find it more reasonable to use parameter estimation from ∂a∂i. Having determined the best demographic models that explain the real data through a likelihood ratio test (Table S1), it is straightforward to use the calculated parameters from these models to estimate the time of the lag phase. Despite the wide confidence intervals, the calculated parameter provides sufficient support for the hypothesis of an ancient introduction involving the lag phase.

We included this new result in our manuscript (L167-L168, L180 – L188, L199-L200, L335 – L337, Fig. 5, and Fig. S8).

The second sentence of your abstract makes it seem like this might be a question you attempt to answer, so I was a little disappointed not to see this already.
R3-2b: We agree with you that this sentence does not correspond to the point we can address. We now replaced the sentence to emphasize that our focus is on the identification of the lag phase (L9-L10).

Besides the above, several details regarding the study require further clarification:

L223: Readers should not have to parse a separate paper to figure out the methods used for the paper they're currently reading.
R3-3: Thank you for bringing up this issue. We have made an effort to provide more information and have clarified that we are providing details on how the dataset was generated in the cited paper (L275-L282).

L224-230: What is the justification for 100kb windows? Also, it would be interesting to see a plot along each autosome (maybe some interesting patterns would emerge).
R3-4: Thank you for your comment. With an assembly size of 385Mb, we have 3,850 windows with 100kb in size. This number of windows is expected to be sufficient for resampling in bootstrapping analyses. The proportion of a single window in the reference genome assembly was mentioned in the manuscript (L285).

We also visualized Tajima's D along the chromosomes (Please refer to Figure 1 in the attached file provided to the reviewer). Tajima's D was predominantly lower than zero in the windows of invasive populations, supporting population expansion.

As you mentioned, we observed an intriguing and very interesting pattern. The Z chromosomes exhibited particularly high Tajima's D in both invasive and native populations, while chromosome 12 also showed increased Tajima's D in native populations. The Z chromosome is known to be a target of selective sweeps in both native and invasive populations (PMID: 36473923, 35477347, and 36368917), and chromosome 12 is similarly targeted in native populations (PMID: 36368917). This observation could be explained by the fact that targets of selective sweeps tend to have increased Tajima's D by removing rare genetic variations in expanding populations.

Despite these interesting patterns, we have opted not to include this result in the manuscript, as the focus of this manuscript is not on selection. Additionally, exploring the association among Tajima's D, demographic change, and selective sweeps would require careful examination through simulation, which is also beyond the scope of this work.

However, we acknowledge the value of these findings and we plan to consider them for future studies.

Thank you again.

L244-L246: Why not perform an actual linkage analysis to determine linkage values for each chromosome rather than this arbitrary selection of snps? This would provide you with an appropriate window size to use for your stats calculations as well.
R3-5: Thank you for your suggestion. This is an excellent suggestion.

Given the absence of a detailed linkage map for the fall armyworm, we opted to assess the effectiveness of our filtering approach by calculating the genomic-average linkage disequilibrium curve. Our analysis revealed that the selection of one SNP within 100bp windows effectively mitigated significant linkage disequilibrium. We inserted the result to the manuscript (Fig. S9 and L311-L312).

Figures 2, 3, 4: Why don't you use the same populations for each analysis (eg, China, French Guiana, Guadeloupe in Fig 2 but not Fig 3; China's not in Figure 4 but it's invasive)? Also, for Figure 2, it seems like there are two individuals lacking heterozygosity (India samples)? Finally, the populations of Brazil, Uganda, and Malawi are listed in the methods but they aren't present in any figure?

R3-6: It would have been ideal to include all populations of mtA and mtB in Figure 2 to generate Figure 3. However, we excluded populations from mtB populations in Puerto Rico and Florida, rice strains from French Guiana and Guadeloupe, and an invasive population from China due to low sample sizes (ranging from two to four). Tajima's D cannot be reliably calculated for these populations. We mentioned this point (L290-L291).

We also observed unusually low heterozygosity in two Indian samples. In the revised manuscript, we explicitly mentioned the results from these two Indian samples and provided a new result displaying the number of homozygous positions for each sample, enabling readers to evaluate the pattern (L112-L115, Fig. S1, and Fig. S2).

Finally, it seems this paper was originally prepared with the methods section placed before the Results and Discussion and not properly adjusted once the Methods were moved after these sections, which leads to a not-so-clear final product. I think a lot of the confusion I encountered could be remedied by placing select details from the Methods section into the Results and Discussion sections. For instance, the different models used for the diffusion analysis are referred to using acronyms in the Results section (where the reader first encounters them), so it is really not clear what the different models are until one gets to the Methods section.
R3-7: We acknowledge that our Results section lacked sufficient detail, particularly concerning model selections. In the revised manuscript, we provided a description of the models used for the model-based diffusion approximation (L171-L179).

Thank you again.

Reviewer #4 (Remarks to the Author):

General comment

In this manuscript, Durand et al. propose to use genomics approaches (instead of data from historical reports) to better identify "lag phases" during biological invasions. For this purpose, they re-analyze a consequent whole genome dataset of 177 Spodoptera frugiperda samples from both native and invasive populations, recently published (Yainna et al. 2022, Scientific Reports). I found the article to be generally well written and to address an interesting question on the dynamics of invasions. The analyses include estimations of genetic diversity, tests for deviation from equilibrium, and demographic inference. I provide several suggestions to clarify between demographics vs. genomics, as well as on methods and presentation of the results that I hope will be helpful for improving the manuscript.
R4: Thank you very much for your constructive review. We appreciate your time for the review. We made our best efforts to address your comments, and we believe that the manuscript is substantially improved now. Thank you again.

My major comment mainly concerns demographic inference. Based on figure 1, I expected the models to include (1) the ancestral admixture (figure 1C), which estimated date is important to address the research question, and (2) the comparison between a reduction of Ne (founder effect) during a given time period followed by an exponential increase in Ne (population expansion; allowing a more ancient introduction date) (figure 1A) versus immediate increase in Ne without founder effect (figure 1B; imposed to known introduction date).
R4-1: Thank you for your comment. This point was also mentioned by other reviewers (R1-1, R1-2, R2-2, and R3-2a).

We agree with you that a realistic scenario of the lag phase should be considered and that the duration of the lag phase needs to be calculated. Since parameter-rich models are required to include an evolutionary scenario involving an invasive population's bottleneck followed by population expansion, we have spent significant efforts to identify a model from which the parameters can be reliably estimated.

Using likelihood ratio tests, we opted for the AM_bottle model, which includes a split between ancestral native and invasive populations with gene flow, followed by the cessation of gene flow (ancestral mixture), and a bottleneck event. From this model, we assessed the time of gene flow cessation (*Ts*) under the assumption that it corresponds to the time of introduction. Despite *Ts* exhibiting wide 95% confidence intervals, it was evident that *Ts* significantly predates the expected timeline in the absence of gene flow (one year). We believe that this result robustly supports the existence of a lag phase.

We inserted the new result (Fig. 5, Table S1, Fig. S6, Fig. S7, Fig. S8, L167 - L168, L171 - L179, L180 - L188, L199 - L200, and L335 - L337).

We also explored the use of the AM_bottleGrowth model, which incorporates exponential population growth following the bottleneck of the introduced population, as you suggested. However, upon evaluation, we found that the AM_bottle model exhibited superior performance in terms of goodness of fit and model complexity (Please refer to Table 1 in the attached file provided to the reviewer) Consequently, we made the decision to exclude the results obtained from the AM_bottleGrowth model. It is possible that exponential population growth may be too recent to accurately calculate the exact growth parameters in this pest species.

I found confusing the choice of samples for the analyses, e.g., including sfR for Ho and Tajima's tests; only Benin (not India) for invasive populations and only mtA (not mtB; but I did not find the information in the methods) for native populations, while excluding Mexico.
R4-2: Thank you for the comments.

The issue of sample selection for Tajima's D was also raised by another reviewer (R3-6). We addressed this concern by excluding groups with a sample size lower than five, as Tajima's D cannot be reliably calculated under such conditions. We added this clarification (see lines 290-291).

Additionally, we excluded the Mexican population from our analysis since the invasive population did not originate from Mexico. This information is also included in the manuscript (see line 70).

Regarding the model-based diffusion approximation, we appreciate your feedback on including the Indian population. Following your suggestion and in response to another reviewer's comment (R1-3), we incorporated the Indian population into our analysis. Upon replicating all analyses using the Indian population, we observed consistent trends between the populations from Benin and India. These results are included in the revised manuscript (Figure 5, Table S1, Figure S7, Figure S8, lines 167-168, 171-179, 180-188, 199-200).

Figures and tables captions also need revision, e.g., I guess the 'sfR' samples in figure 2 and figure 3 correspond to the rice strain; what do the different panels correspond to in figure 3; expand scenario codes in table 1.
R4-3: Thank you for pointing out this issue. We fixed the strain names in Figure 2 and Figure 3. We also added the full name of the models in the table legend (Table S1).

Specific comments

Keywords: add relevant keywords about wgs data
R4-4: This is a good suggestion. I added ‘invasive genomics’ (L22).

L.43: which component of diversity?
R4-5: We excluded ‘hyperdiverse’ here because this word does not seem to be necessary (L28).

L.47-49: I agree that time for adaptive evolution has been proposed to explain the lag times between introduction and spread. But one common explanation is a demographic issue (negative density dependence).
R4-5: This is an excellent suggestion. Thank for this suggestion. We added this possibility (L34-L37).

L.55-57: In addition to this consideration, historical records are largely incomplete for most invasive species.
R4-6: We absolutely agree with you. We added the corresponding sentence (L45).

L.58-61: Clearly explain the link in between here. For example, genomic data combined with demographic modeling help deciphering the geographic origin (spatial sampling) or whether the current population is a daughter population of early-detected propagules (temporal sampling); changes in effective population size (Ne) inferred from genomic data can reveal a reduction in Ne followed by an increase in Ne, which can reflect the impact of demographic changes (founder event, population expansion/migration, respectively); etc. This can be clarified providing example studies.
R4-7: Thank you for bringing up this issue. We elaborated on the logic with a specific focus on the lag phase and a citation showing examples (L46-L53).

We did not mention temporal sampling, as this sampling remains very challenging in many cases even before the start of population genomics analysis.

L.62-63: I do have a question regarding “the socio-ecological importance of identifying lag phases in invasive species (L.61-62)”, because “it generally corresponds to the last opportunity to eradicate an alien pest species (L.13-14)". Considering a study that 1) manages to collect samples just a few generations after an introduction; 2) performs sequencing and analysis of genomic data to determine whether (or not) and when such an initial “lag phase" has occurred; 3) reveals changes in Ne over time that could be indicative of a lag phase (see my comment on L.58-61); what are the potential implications in terms of population management? Do the studied populations still have the same demographic status? For example the present study: 6 years after collection so potentially 60 generations based L.137.
R4-8: Thank you for bringing up this issue. We recognize it as an excellent point, and it has prompted us to contemplate the usefulness of genomics approaches in reducing the cases of invasion.

The identification of lag phases through genomics analysis may not alter the circumstances of the deleterious effects caused by extant invasive pest species. However, this identification will provide valuable information for evaluating the current implementations of pest management and biosecurity plans. Consequently, studies on lag phases may contribute significantly to preventing future invasions

We added this logic (L53-L55).

L.83: Delete the space after the opening parenthesis.
R4-9: We fixed this error (L68). Thank you.

L.75-89: There are a couple of studies described here, the results of which are very important because they correspond to the knowledge upon which the hypotheses of the study are based. However I don't find that Figure 1 is sufficiently explicit. I suggest the authors to show a multi-panel figure summarizing (1) the sampling, (2) the results of previous studies, and (3) the competing scenarios of Figure 1A and 1B, which should be the ones tested in demographic inference (figure S1).
R4-10: Thank you very much for this suggestion. We acknowledge that the hypothesis was not clearly displayed in Figure 1.

In the Figure 1 legend, we explicitly stated that Fig 1C pertains to the summary of previous studies. Additionally, we modified Fig. 1C to illustrate the hypothesis we aim to test based on these studies.

We decided not to display the results of Tay et al. (2022) in the figure because our study did not test the hypothesis that the extant invasive population originated from the sample intercepted from China to Australia.

L.85-89: Reformulate.

All invasive populations show a common pattern of admixture between native mtA and mtB strains, suggesting a common origin [...]. If these repeatedly observed patterns are true, it is tempting to hypothesize that the introduction of FAW in Benin is more ancient than currently thought. The homogenization of genome sequences after the admixture event is unlikely to have emerged over the course of just a few generations (~10 generations). Therefore, a founding admixture in Benin (or India) is expected to have occurred (and thus the introduction of FAW) before the detection date of the species (2016) because of the existence of a lag phase where the species remained undetected (i.e., too low population size). Population genetic variation can be used to test for the impact of a bottleneck during introduction (but not necessarily true due to the admixture event) and of a subsequent recent expansion. Furthermore, we should observe incongruence between the time of the admixture event and the time of detection.

Following this hypothesis, the invasive population in Benin (or India) initiated all other introductions worldwide? I want to stress that the discussion L.184-187 on the fact that the ancestral admixture might have occurred in an unknown location within the native range, from which populations were independently introduced to Africa and Asia, has to remain in future versions of the manuscript.
R4-11: You are absolutely right. Since we do not know exactly when and where the admixture occurs, it is challenging to link the admixture to lag phase.

Rather, we observed that the observed whole-genome differentiation between invasive and native populations provides sufficient motivation to test for a lag phase. Without a lag phase, we need to assume that the observed genomic differentiation can occur within less than one or two years, which is the duration between the initial report of invasion and the sampling date. It is unlikely that this time frame is sufficient to result in differentiation much higher than that observed among all pairs of native populations, including the USA and Argentina.

We rewrote this part to mention that the observed whole-genome differentiation between native and invasive populations cannot be explained without considering the existence of a lag phase (L77-L82).

L.102: repetition of 'statistical'
R4-12: Thank you for pointing out this error. We fixed it (L95).

L.103: Are populations in Benin and India genetically differentiated? i.e., is it possible to differentiate these scenarios: (1) founding admixture between mtA and mtB in Benin then spread to other invaded areas, (2) founding admixture in India then spread to other invaded areas, (3) ancestral admixture (ghost population) then independent introductions in Benin and India?
R4-13: Thank you very much for pointing out these possibilities. We did not detect any discernible differences between these two populations, with the exception of two Indian samples (Fig. 2B in Yainna *et al.,* PMID: 36473923). Thus, it appears impossible to test these possibilities using genetic analysis.

The invasion of fall armyworms was reported from Benin and India in 2016 and 2018, respectively. Therefore, we think that, as far as the analyzed samples are concerned, Indian samples arrived from African populations. Since this scenario is highly speculative, we decided not to mention it in the manuscript.

L.113: I suggest to provide the distribution of individual observed heterozygosity by population (and provide this figure 2 in supplementary material). This will help follow the text on comparisons between native/invasive populations.
R4-14: Thank you for your suggestion. We included a violin plot depicting heterozygosity by populations (L111-L112 and Fig. S1).

L.114: Also in figure 2, there is one sample with higher heterozygosity. Is this sample admixed with another population?
R4-15: Thank you very much for this comment. To address this comment and also in response to R4-24 (please see below), we conducted a test to assess whether admixture could significantly increase heterozygosity. We observed that admixture could increase heterozygosity only approximately 1%. This result is included in the manuscript (L116-L122 and Fig. S3).

As a result, we did not explicitly mention the possibility that admixture could increase the heterozygosity of PR19, although the observed pattern itself is mentioned (L113-L114).

L.115: “moderate decline or due to an increase after bottleneck”. Did the authors test for the impact of a founder effect and subsequent increase in effective population size?
R4-16: We acknowledge that the sentence was not clearly written. We revised it (L123-L126).

L.120: Tajima’s D average estimates are negative for invasive populations in Benin and India, suggesting these populations are expanding. Are values of Tajima’s D similar in native and invasive populations?
R4-17: Thank you for this suggestion. Now, we included a statistical test to compare Tajima’s D between invasive and native populations (L138-L140).

L.125: Increase font size for sample IDs. Are these two individuals from India (with lower pop-specific SNVs) also those with almost completely homozygous genomes in figure 2? What is the coverage for these two samples?
R4-18: Thank you for pointing out this issue. It was addressed by another reviewer (R3-6). We also find it peculiar. We added additional information including the sequencing coverage and the number of homozygous positions to ensure that readers can evaluate the result (Fig. S1 and L111-L115). We also increased the font size of Figure 2.

L.135-136: The authors assume that a cessation of gene flow would be "indicative" of the existence of a lag phase. Can processes other than cessation gene flow leave the same signature in the SFS? I agree that 10 generations can be a little short to obtain genetic differentiation between native and

invasive populations from the accumulation of independent mutations/genetic drift due to geographic isolation, especially if the initial effective population size was large. From a highly diverse admixed population, could a founder effect of medium intensity result in the observed patterns of heterozygosity?
R4-19: Thank you for your comment, and we agree with the points.

1) In the revised manuscript, we explicitly estimated the time of introduction to test the lag phase. We believe that this approach is more robust than relying solely on model selection because it eliminates the need to assume that the cessation of gene flow indicates the presence of a lag phase. As the response to R4-1, we added this result (Fig. 5, Table S1, Fig. S6, Fig. S7, Fig. S8, L167-L168, L171-L179, L180-L188, L199-L200, and L334-L336).

2) We also agree with the second point, which was also mentioned by other reviewers (R2-1a, R2-1b, and R3-1). We conducted coalescence simulations to test whether the negative sign of Tajima's D could result from the introduction of numerous insects. Our observations indicate that the negative sign of Tajima's D cannot be generated unless far more than 1,000 insects are introduced simultaneously. We included this new result (Fig. S4, Fig. S5, L126-L135, and L144-L153).

L.138-143: Provide the full name of scenarios at first use, especially because of the format of the article (methods at the end). More generally, the authors may consider having a sentence summarizing the methods at the beginning of each paragraph.
R4-20: Thank you for pointing out this issue. We provided detailed information (L171-L179).

L.140: Table 1. Why only testing for Benin and not India?
R4-21: Please see the response to R4-2.

L.147-148: see my comment on L.120.
R4-22: Thank you for this comment. As mentioned before (R4-17), we performed a statistical test to compare Tajima's D between invasive populations and non-Mexican populations, and the result is now included in Result (L138-L140).

Since this comparison is not the key finding in our study, we did not mention this result in the Discussion to make the point of this manuscript clear.

L.169: "Lag phases explain the genetic paradox of invasion": ?
R4-23: Thank you for your comment. We added an explanation of the 'genetic paradox of invasion' here (L216-L217).

L.178-180: I find this discussion here about bottlenecks a bit confusing given that previous studies have demonstrated an ancestral admixture common to all invasive populations and that the present study, in agreement, supports very slight reduction in heterozygosity.
R4-24: Thank you for your comment. This point was mentioned by other reviewers (R1-7 and R2-1c).

Indeed, the possibility that genetic admixture has increased genetic diversity should be discussed. Therefore, we estimated the extent to which genetic admixture could contribute to heterozygosity in invasive populations. We observed that mtA and mtB specific variations account for 8.63% - 11.8%% and 0.902% - 1.31% of genetic variations in invasive populations, respectively, implying that genetic admixture can increase the genetic diversity at most to around 1% in the invasive population. We inserted this result and corresponding logic (Fig. S3 and L116-L122).

L.180-181: What was is the inferred duration of the lag phase? The authors do not provide or discuss population parameter estimates from demographic inference (posterior distributions for best scenario). Why?
R4-25: Please refer to the response to R4-1.

L.188: mtA only in the native pools of demographic models? Justify. Could the results be impacted by an unmodelled demographic event (e.g., ancestral admixture, expansion)?
R4-26: Thank you for pointing out this issue. mtA should be removed in the sentence (L237). We also mentioned the limitation of model-based demographic inference (L259-L260).

L.228: population structure
R4-27: Thank you for this comment. We fixed the sentence (L288).

L.229: ‘average Tajima’s D over windows’
R4-28: Thank you for this comment. We rephrased the sentence (L289).

L.256-261: Provide the prior distribution for population parameters.
R4-29: The initial parameters were determined randomly. We inserted this information (L333).

Reviewers' comments:

Reviewer #2 (Remarks to the Author):

I am generally satisfied that the authors have addressed my comments.

Regarding the response to my first comment, the authors should justify their choice of simulation parameters. For example, what is the justification for a native population size of 106 before a bottleneck? This number would ideally be based on an empirical estimate of historical Ne from native-range samples.

Regarding the effect of invasive-range admixture on Tajima's D: I'm not sure this is the right approach for quantifying the extent of admixture, especially if the authors are not certain that there is no gene flow between the mtA and mtB lineages in the native range. Even a small degree of gene flow in the native range would remove a large proportion of fixed differences between lineages, even if allele frequencies remained highly divergent between lineages. So if there is any inter-lineage gene flow in the native range, lineage-specific variants will be a poor measure of the extent of admixture in the invasive range.

If I understand correctly, the authors claim that because non-native samples typically carry ~1% of alleles unique to the mtB lineage (& ~10% from mtA), admixture resulted in a ~1% increase in heterozygosity in the non-native population. This conclusion is not obvious to me, though the line of reasoning is a little hard to follow because the Methods do not describe exactly how the proportion of mtB and mtA alleles were calculated per invasive sample. In any case, alleles with frequencies that are divergent when comparing mtA and mtB samples, but which are not fixed differences, will surely contribute to heterozygosity in an admixed population quite substantially.

Minor comments (line numbers referred to resubmitted manuscript without markdown)

L16: "even much lower" should read "even lower"

L115: two periods after "heterozygosity"

L133: ",We assumed that …" unnecessarily capitalised

Reviewer #4 (Remarks to the Author):

I would like to thank the authors for the detailed responses to the reviewers' comments. The context of the study is well presented, and the authors stated clear goals. The study now presents a more robust investigation of a lag phase during the fall armyworm invasion - the authors performed coalescent simulations to compare observed and simulated patterns under various assumptions (changes in effective population size and gene flow). This study will be a nice addition to previous studies using genomics to better understand certain aspects of invasion dynamics.

I have a few additional comments:

The previous version of this manuscript focused only on the Benin invasive population, which is still apparent in some paragraphs. For example, L96: "samples collected from Benin and India in 2017, shortly after the invasion was reported in 2016". Here the authors provide detection date in Benin and

cite the paper of Goergen and colleagues (first report in Benin; ref. 25). What about the India invasive population? Genetic data was obtained from samples collected in 2018, a few weeks/months(?) after the detection was reported in May 2018? Please revise the text and include citation Sharanabasappa et al. 2018. Consider also revising L309-312.

In Figs S4 and S5 (also L304): for the bottleneck event, it may be more relevant to specify 10 generations before genetic sampling. Could the authors also justify the 10 generations value? If the bottleneck duration corresponds to the time between first report and genetic sampling, it should be one year for Benin, thus 12 generations considering a generation time of one month. However, for the samples from India, is this number of generations consistent (see my previous comment)?

L186-188: The time since cessation of gene flow is somehow a function of effective population sizes. Although the models involve different source populations, the estimated parameters in Fig S8 show: 1) an ancient divergence and high founding diversity (Florida); 2) a relatively ancient divergence and a bottleneck of intermediate intensity (Puerto Rico); 3) a recent divergence followed by extreme bottleneck (Mississippi). Could increasing the intensity of the bottleneck (the value of the reduction factor b) make it possible to get a Ts closer to 1 year?

L262-272: The authors argue that genomic studies documenting lag phases in invasion will contribute to the management of pest species. Their finding implies that the sampled introduced populations were present before first historical report of the species, and that population recovery might explain the failure of preventing fall armyworm invasion. If I understand well, the authors thus encourage monitoring programs for early detections and systematic report.

Table 1: Degree of freedom and D-statistics are not presented.

Figs 5 and S8: Could the authors expand the parameter abbreviations in the legend or directly in the axis label.

Figs S4 and S5: “population size” should be “effective population size”.

Reviewers' comments:

Reviewer #2 (Remarks to the Author):

I am generally satisfied that the authors have addressed my comments.
R2: Thank you for your positive review. We further attempted to improve the manuscript according to your suggestion.

Regarding the response to my first comment, the authors should justify their choice of simulation parameters. For example, what is the justification for a native population size of 106 before a bottleneck? This number would ideally be based on an empirical estimate of historical Ne from native-range samples.
R2-1. Thank you for your comment. We agree that it is important to use a justifiable parameter for the initial population size. We calculated the average heterozygosity for each native sample, followed by calculating the average population size. The effective population size was then determined using the classical formula, Heterozygosity = 4 × effective population size × mutation rate. The simulation showed an identical trend, and we updated the results and method in the revised manuscript (Figure S4, Figure S5, L146-L150, L150-L157, L171-L172, L172-L174, L319-L323.

Regarding the effect of invasive-range admixture on Tajima's D: I'm not sure this is the right approach for quantifying the extent of admixture, especially if the authors are not certain that there is no gene flow between the mtA and mtB lineages in the native range. Even a small degree of gene flow in the native range would remove a large proportion of fixed differences between lineages, even if allele frequencies remained highly divergent between lineages. So if there is any inter-lineage gene flow in the native range, lineage-specific variants will be a poor measure of the extent of admixture in the invasive range.
R2-2: Thank you for your comment. We are absolutely agree with you that Tajima's D cannot be used to measure admixture, and we have not used Tajima's D for this purpose.

We acknowledge that our writing might have caused misunderstanding, especially by this sentense: 'Furthermore, a largely uniform distribution of mtA or mtB-specific SNVs across all invasive individuals suggests that genetic admixture between mtA and mtB alone is unlikely to cause the observed negative sign of Tajima's D' in the previously submitted manuscript (L193-L195).

We removed this sentence to avoid potential misunderstanding (L217).

If I understand correctly, the authors claim that because non-native samples typically carry ~1% of alleles unique to the mtB lineage (& ~10% from mtA), admixture resulted in a ~1% increase in heterozygosity in the non-native population. This conclusion is not obvious to me, though the line of reasoning is a little hard to follow because the Methods do not describe exactly how the proportion of mtB and mtA alleles were calculated per invasive sample. In any case, alleles with frequencies that are divergent when comparing mtA and mtB samples, but which are not fixed differences, will surely contribute to heterozygosity in an admixed population quite substantially.
R2-3: Thank you for your comment. We agree that non-fixed genetic variations could substantially increase the genetic diversity of hybrids. Knowing this effect, we already included both fixed and polymorphic variations specific to mtA or mtB. We attempted to clarify this point (L134-L135).

Minor comments (line numbers referred to resubmitted manuscript without markdown)

L16: "even much lower" should read "even lower"
R2-4: Thank you for your comment. We fixed the sentence (L20)

L115: two periods after “heterozygosity”
R2-5. Thank you for this comment. We fixed it (L130).

L133: “,We assumed that …” unnecessarily capitalised
R2-6. Thank you for your comment. We fixed the sentence (L155)

Reviewer #4 (Remarks to the Author):

I would like to thank the authors for the detailed responses to the reviewers' comments. The context of the study is well presented, and the authors stated clear goals. The study now presents a more robust investigation of a lag phase during the fall armyworm invasion - the authors performed coalescent simulations to compare observed and simulated patterns under various assumptions (changes in effective population size and gene flow). This study will be a nice addition to previous studies using genomics to better understand certain aspects of invasion dynamics.

I have a few additional comments:
R4: Thank you very much for your comment. We are pleased that you find our manuscript improved. We made further revisions according to your excellent suggestions.

The previous version of this manuscript focused only on the Benin invasive population, which is still apparent in some paragraphs. For example, L96: "samples collected from Benin and India in 2017, shortly after the invasion was reported in 2016". Here the authors provide detection date in Benin and cite the paper of Goergen and colleagues (first report in Benin; ref. 25). What about the India invasive population? Genetic data was obtained from samples collected in 2018, a few weeks/months(?) after the detection was reported in May 2018? Please revise the text and include citation Sharanabasappa et al. 2018. Consider also revising L309-312.
R4-1: Thank you for this comment. We understand the importance of identifying the exact sampling year when the Indian samples were collected. For this purpose, first of all, we emailed the corresponding author of the paper and received the following response:

‘Dear Ki-Woong
The samples were collected in 2018 not 2017.
Thank you’

This response confirms that the sampling year is 2018. In the manuscript, we cited this paper with a clear description of the sampling year (L110-L112 and L338-L339).

In Figs S4 and S5 (also L304): for the bottleneck event, it may be more relevant to specify 10 generations before genetic sampling. Could the authors also justify the 10 generations value? If the bottleneck duration corresponds to the time between first report and genetic sampling, it should be one year for Benin, thus 12 generations considering a generation time of one month. However, for the samples from India, is this number of generations consistent (see my previous comment)?
R4-2. Thank you for this excellent comment. We agree with you that we need to consider a different number of generations for the Indian samples, which were collected in 2018. Therefore, we performed additional simulations showing Tajima’s D after 20 generations of bottleneck. The results exhibited the same trend as observed between 10 and 20 generations ago. We inserted this new result (Figure S4, Figure S5, L143-L146, L150-L157, and L172-L174).

L186-188: The time since cessation of gene flow is somehow a function of effective population sizes. Although the models involve different source populations, the estimated parameters in Fig S8

show: 1) an ancient divergence and high founding diversity (Florida); 2) a relatively ancient divergence and a bottleneck of intermediate intensity (Puerto Rico); 3) a recent divergence followed by extreme bottleneck (Mississippi). Could increasing the intensity of the bottleneck (the value of the reduction factor b) make it possible to get a Ts closer to 1 year?
R4-3: You are absolutely right that *Ts* and *b* are dependent parameters. We performed the estimation of *Ts* and *b* using maximum likelihood. In other words, our results show that, according to the real data, *b* cannot be so small as to cause *Ts* to be closer to 1 year. We believe that confusion might have arisen because we did not mention that ∂a/∂i is based on a maximum likelihood framework, which is a process of finding the a set of parameters best explaining real data by maximizing likelihood. Therefore, we clarified that we used a maximum likelihood framework for parameter estimation (L191 and L336).

L262-272: The authors argue that genomic studies documenting lag phases in invasion will contribute to the management of pest species. Their finding implies that the sampled introduced populations were present before first historical report of the species, and that population recovery might explain the failure of preventing fall armyworm invasion. If I understand well, the authors thus encourage monitoring programs for early detections and systematic report.
R4-4: Thank you for this excellent comment. We have inserted a sentence to highlight the importance of monitoring and systematic reporting to prevent pest invasions (L293-L295).

Table 1: Degree of freedom and D-statistics are not presented.
R4-5. Thank you for pointing out the missing information. We have added the degrees of freedom and D-statistics in Table S1.

Figs 5 and S8: Could the authors expand the parameter abbreviations in the legend or directly in the axis label.
R4-6. Thank you for this comment. Although we referred to Figure S6 for the description of parameters in the legend of Figure S8, we acknowledge that it would be better to describe the parameters again here. Therefore, we added the explanation of each parameter in the legend of Figure S8.

We also noted that the legend of Figure 5 already includes the description of a parameter shown in the figure (L421-L422).

Figs S4 and S5: “population size” should be “effective population size”.
R4-7. Thank you for this comment. We fixed the legends.