nature portfolio

Peer Review File

A comparative genomic analysis at the chromosomal-level reveals evolutionary patterns of aphid chromosomes

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Huang et al. sequenced a Macrosiphini aphid to explore the mechanisms of the rapid rate of chromosomal rearrangements in aphids. Specifically, recent literature has shown that aphids have a relatively extreme rate of chromosomal rearrangements (inter and intra) and the authors set out to explore this further with new chromosomal genome assembly.

Overall, I love the topic because I am also interested in this, albeit to a lesser degree. I thought the authors did a great job with the analyses; however, there were a few things that were missing or that stood out. First, there is no information about the aphid species, where it was collected, when, etc. This is incredibly important because I reviewed a recent paper on aphid genomes and the authors sequenced a different aphid than what the paper was discussing. This information, in addition to how the authors identified the species is crucial in order for me to believe they actually sequenced a Semiaphis species. Lastly, and most importantly, I looked for the data on GenBank and can not find any listed in the manuscript. In order for me to properly confirm what the author did, I need to see it and examine it, but I could not. That said, the GitHub repo is viewable and the scripts look like they are in working condition although I did not test them.

Next, some of the sentences seemed odd and out of place. I believe the authors need to carefully go through the manuscript and correct it accordingly as best as possible.

Lastly, one thing that stands out to me is the sample size. The Macrosiphini include thousands of species; therefore, I think their discussion should consist of some discussion about experimental design in order to identify these mechanisms. For example, they do not have chromosomal-level genomes for all species of a genus; therefore, they have very little of the evolutionary history of chromosomal evolution. Moreover, just snapshots of chromosomes over the course of ~100 million years of aphid evolution, which suggests not a clear picture whatsoever.

Reviewer #2

(Remarks to the Author)
This manuscript performed a serial analysis and tried to understand the evolutionary patterns of aphid chromosomes. Evolution of aphid chromosomes, indeed, is an intriguing question in the field. This manuscript made efforts to step forward to peer into the question.

I noticed that the authors tried to move beyond stories of the green-peach aphid and pea aphid (ref 9 and 45). However, the logic of this story is pretty weak. The main issue is that the hypothesis is not clear. There is no obvious conclusion generated by the comparative analysis. The manuscript is written descriptively. The story is mixed with the genome sequence of S. heracleid and an overview of aphid chromosome evolution. It is very difficult to understand the logic.

In the Introduction, they mentioned “incorporate more chromosomal-level aphid genomes”, then they sequenced the genome of S. heraclei. Actually, many chromosomal-level aphid genomes have already been published, such as Mathers et al. 2022 (Reference 32). With those high-quality genomes, I believe that comparative genomic analysis can be achieved. So, the reason for sequencing S. heraclei is very weak. The uniqueness of S. heraclei is not addressed enough in the manuscript. S.heraclei is a notorious pest; its high-quality genome will be an important resource to understand this pest. In my opinion, the writing will be much better if it is focused on the biology of S.heraclei.

Minor:
1. page 2, line 46, ref 9 is cited inappropriately.
2. Page 2 line 47 “…. 23 subfamilies” lack a reference.
3. The Introduction should include “Chromosome rearrangement and TE”. Ref 70 is a very good paper for TE and gene family evolution.
4. Page 5, line 123, “no inter-autosomal rearrangements”. “no obvious inter-autosomal rearrangements were detected” may be better.
5. “Chromosomal rearrangement rates”, what it is and how it is calculated?

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
The authors have addressed all my concerns. The paper looks very good now.

Few minor things for them to further improve the quality of paper.
1. Abstract. The coherence in the abstract can be improved. The sentences from 29-32, need to be adjusted in order to fit with the context.
2. Please check the tense. When you describe the results, it is better in the past tense. for example, line 151 “we speculate” should be “we speculated”, line 152 “…is mainly,” should be “was mainly”. Line 156 “are generally”, should be “were generally”, etc. Please check the entire paper, which is scientifically amazing now.
3. Fig 2a, are there two X chromosome for E.lanigerum and three for S.chinensis? It looks very weird.

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

Comment 1: Huang et al. sequenced a Macrosiphini aphid to explore the mechanisms of the rapid rate of chromosomal rearrangements in aphids. Specifically, recent literature has shown that aphids have a relatively extreme rate of chromosomal rearrangements (inter and intra) and the authors set out to explore this further with new chromosomal genome assembly.

Overall, I love the topic because I am also interested in this, albeit to a lesser degree. I thought the authors did a great job with the analyses; however, there were a few things that were missing or that stood out. First, there is no information about the aphid species, where it was collected, when, etc. This is incredibly important because I reviewed a recent paper on aphid genomes and the authors sequenced a different aphid than what the paper was discussing. This information, in addition to how the authors identified the species is crucial in order for me to believe they actually sequenced a *Semiaphis* species. Lastly, and most importantly, I looked for the data on GenBank and can not find any listed in the manuscript. In order for me to properly confirm what the author did, I need to see it and examine it, but I could not. That said, the GitHub repo is viewable and the scripts look like they are in working condition although I did not test them.

Response 1: We sincerely appreciate your careful evaluation and positive comments on our manuscript. First, we acknowledge that the sampling information for *Semiaphis heraclei* (the celery aphid) was indeed limited and lacked detail. To address this, we have added the following information in the Sample collection and sequencing section (line 473-478): "The parthenogenetic wingless adult and nymphal aphids of *Semiaphis heraclei* were collected in May 2019 from a planting base of Lonicera japonica (variety: 'Siji jinyinhua') located in Zhengcheng Town, Pingyi County, Shandong Province, China (35°16′0″N, 117°38′56″E). In the laboratory, the aphids were reared on cuttings of Lonicera japonica for over 10 generations under controlled conditions (temperature: 25±1°C, relative humidity: 70% ± 5%, light cycle: 14 h light : 10 h dark). The aphids were identified by Associate Researcher Jiang Liyun at the Institute of Zoology, Chinese Academy of Sciences, and the specimens are stored at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences."

Additionally, we have submitted the sequencing data and genome assembly to the NCBI database (Genome accession: JBEIVQ000000000; Raw sequences are available at the Bioproject accession: PRJNA1061761). Previously, this data was not accessible, but it has now been fully released.

Comment 2: Next, some of the sentences seemed odd and out of place. I believe the authors need to carefully go through the manuscript and correct it accordingly as best as possible.

Response 2: Thank you for providing so many valuable suggestions for our work. We have made effort to address them and have responded to each one below.

Comment 3: Lastly, one thing that stands out to me is the sample size. The Macrosiphini include thousands of species; therefore, I think their discussion should consist of some discussion about experimental design in order to identify these mechanisms. For example, they do not have

chromosomal-level genomes for all species of a genus; therefore, they have very little of the evolutionary history of chromosomal evolution. Moreover, just snapshots of chromosomes over the course of ~100 million years of aphid evolution, which suggests not a clear picture whatsoever.

Response 3: Thank you for your suggestions. Our manuscript lacked a discussion on the limitations of the study, so we have added the following content to the discussion (line 430-437):" It is noteworthy that, compared to the over two thousand species within the Macrosiphini tribe, the number of published chromosome-level genomes remains limited. High-quality chromosome-level genomes with broad taxonomic representation are still lacking, hindering our ability to fully unravel the chromosomal evolution history of the Macrosiphini tribe. Furthermore, there is a significant gap in the availability of genome assemblies for the other subfamilies of aphids except Aphidinae, which obstructs aphidologists from gaining a comprehensive understanding of aphid chromosomal evolutionary history.

Nevertheless, the genomes we selected are representative in terms of taxonomy, covering the major branches of the Macrosiphini tribe, which provides a foundation for understanding the karyotypic evolution of the entire tribe. Furthermore, with the advancement of sequencing and assembly technologies, more high-quality chromosome-level genomes will be generated in the future, thereby addressing the current limitations. We believe that the current study lays the groundwork for future work and has revealed some important patterns of karyotypic evolution, which provide direction for future research.

Minor:

Introduction

Comment 4: Lines 60-61, 'However, due to…': This sentence is untrue because there are more than three chromosomal-level genomes publicly available for some time.

Response 4: Thank you for your valuable feedback. We understand your concern regarding the statement about the availability of chromosomal-level genome assemblies. To clarify, the sentence in question refers specifically to the study by Mathers et al., which was indeed limited to three aphid species due to the availability of chromosomal-level genome assemblies at the time of their research. We have revised the sentence for better clarity (line 69-70):

"However, Mathers et al.'s study was confined to only three aphid species, due to the limited availability of chromosomal-level genome assemblies at the time."

We believe this revision clarifies our intent while acknowledging the broader availability of genomic resources. Thank you again for your valuable feedback.

Comment 5: Line 61, 'We': who are you referring to when using, "We"?

Response 5: Thank you for your helpful comment. In the original sentence, 'we' refers to the broader research community or the scientific community working in aphid genomics. To avoid any confusion, we have revised the sentence to clarify this point (line 71-74): " Incorporating more chromosomal-level aphid genomes into genomic evolutionary analysis is necessary to explore the mechanisms and pattern behind chromosomal rearrangement in aphids, investigate whether different aphid lineages exhibit varying rates of chromosomal rearrangement and the reasons behind these differences, and to determine whether a higher rearrangement rate might lead to an increased speciation rate."

Comment 6: General comment: You do not have any biological information about the aphid species you sequenced. I find that odd and I think you should put information about it and it's natural history into the introduction.

Response 6: Thank you for your valuable comments. We have added biological information about *Semiaphis heraclei* in the introduction, including its geographical distribution, major host plants, and the basic characteristics of secondary metabolites produced by these host plants (line 75-83), which were shown in the Response 1 section. Additionally, we briefly introduced gene families involved in detoxification and adaptation to plant secondary metabolites in aphids, including cytochrome *P450* monooxygenases (*P450s*), carboxyl/cholinesterases (*CCEs*), UDP-glucosyltransferases (*UGTs*), and glutathione S-transferases (*GSTs*), emphasizing the role of these genes in host adaptation in aphids (line 83-88). Furthermore, we have incorporated a section (line 326) in the manuscript that compares the aforementioned gene families across different aphid species to explore the genetic mechanisms underlying the host adaptation and evolutionary processes in *Semiaphis heraclei*.

Materials and Methods

Comment 7: General comment: You are also lacking quite a bit of information regarding the collection of the species. For example, where did you collect it? On what host plant? How did you identify it?

Where did you deposit voucher specimens? What did host did you culture the aphid on? What were the culture conditions (e.g., photoperiod, temperature, etc.)? This is mainly for researchers that would like to go back and further study your organism and system.

Response 7: Thank you for your key suggestions. We have added relevant information on species collection to the sample collection and sequencing section (line 472-477)：“The parthenogenetic wingless adult and nymphal aphids of Semiaphis heraclei were collected in May 2019 from a planting base of Lonicera japonica (variety: 'Siji jinyinhua') located in Zhengcheng Town, Pingyi County, Shandong Province, China (35°16′0″N, 117°38′56″E). In the laboratory, the aphids were reared on cuttings of Lonicera japonica for over 10 generations under controlled conditions (temperature: 25±1°C, relative humidity: 70% ± 5%, light cycle: 14 h light : 10 h dark). The aphids were identified by Associate Researcher Liyun Jiang at the Institute of Zoology, Chinese Academy of Sciences, and the specimens are stored at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences.”

Comment 8: Line 415: change ‘Contig’ to ‘contig’

Response 8: Thank you for your suggestion. We have revised it in the revised manuscript (line 491).

Comment 9: Line 415, ‘We checked the…’: what taxon databases did you use in BlobTools to check for contamination? This information is important because it conveys you know what the potential contaminants are for an aphid considering its biology.

Response 9: In the process of using BlobTools to detect contamination sequences in the genome assembly, we used the complete Nucleotide Sequence Database, NCBI taxdb and taxdump, which encompass almost all nucleotide sequences submitted to NCBI. This additional information has been included in the revised manuscript to provide clarity to the audience (line 493).

Comment 10: Lines 424-425, ‘We identified repeats…’: I am not sure how you identified repeats using the ‘same pipeline’, but you haven’t discussed a pipeline yet. This sentence seems really odd and out of place.
Response 10: Thank you for pointing this out. In the revised version, we added a detailed description of the analysis pipeline used in the revised manuscript (line 501-509), to make this section clearer and more coherent.

Comment 11: Line 424, ‘…include another eight…’: I think you want to use ‘including’.
Response 11: Thank you for your suggestion. We have revised it in the revised manuscript (line 501).

Comment 12: Line 431, ‘were’: please change to ‘was’
Response 12: Thank you for your suggestion. We have revised it in the revised manuscript (line 510).

Comment 13: Line 433, ‘homologous species’: I am not sure what a homologous species is; therefore, this may be a typo.
Response 13: Thank you for your observation. The term ‘homologous species’ was indeed unclear. We have revised it to ‘closely related species’ to enhance clarity and accurately reflect the intended meaning (line 512). The revised version is now clearer and more precise.

Comment 14: Line 444-445, ‘…the other eight chromosomal-level…’: why did you annotate the other genomes again if they have annotations publicly available? What assemblies did you use? Lots of information is missing here that is important to your audience.
Response 14: Thank you for your valuable feedback. The number of genes identified can vary significantly with different annotation strategies. In our study, we needed to compare the number of protein-coding genes across these aphids. To ensure consistency and accuracy in annotation, we therefore annotated these genomes using the same pipeline. The eight re-annotated aphid genome assemblies at the chromosome level refer to the species mentioned in the introduction that were used for comparative analysis (line 93-96). Detailed information about these assemblies is provided in Supplementary Table 4 and 12. This additional context has been included in the revised manuscript to provide clarity to the audience.

Comment 15: Line 448, “We used protein-coding…”: in order to repeat your study, readers will again need the assemblies you used, citations, and links to the data. ALL of this information is crucial to reproduce your study.
Response 15: Thank you for your valuable feedback. We have added citations and download links for the data in Supplementary Table 4. Additionally, the nine chromosome-level aphid genomes and their annotation files used for comparative analysis have been shared on Figshare (link: 10.6084/m9.figshare.27314556). We hope this provides readers with the necessary resources to reproduce our study.

Discussion
Comment 16: Line 332, ‘after involved more lineage sampling’: change to ‘increasing the lineage

sampling'

Response 16: Thank you for your suggestion. We have revised it in the revised manuscript (line 377).

Comment 17: General comment: You combine your rearrangement data with a phylogeny of aphids that were randomly sampled from 5000+ species. Many of your most recent common ancestors of the terminals in your phylogeny are estimated to be 10's of millions of years old. Maybe to get at the precise mechanisms you should sample aphids that share a most recent common ancestor over a shorter geologic timeframe, while sampling all known taxa. It seems the discussion is a lot of speculation that boils down to experimental design and taxon sampling.

Response 17: Indeed, due to the limited availability of chromosome-level aphid genomes, the aphid species used for analyzing chromosomal rearrangements are relatively distantly related. To mitigate this limitation, we focused exclusively on calculating the chromosomal rearrangement rates between aphid species with closer phylogenetic relationships, rather than comparing rates across more distantly diverged tribes or subfamilies. Additionally, we have included a discussion on the limitations of our study in the discussion section (line 430-436): "It is noteworthy that, compared to the over two thousand species within the Macrosiphini tribe, the number of published chromosome-level genomes remains limited. High-quality chromosome-level genomes with broad taxonomic representation are still lacking, hindering our ability to fully unravel the chromosomal evolution history of the Macrosiphini tribe. Furthermore, there is a significant gap in the availability of genome assemblies for the other subfamilies of aphids except Aphidinae, which obstructs aphidologists from gaining a comprehensive understanding of aphid chromosomal evolutionary history. Addressing this issue requires more available chromosome-level aphid genomes in the future."

Comment 18: Lines 386-387, "Gene loss…": Are these genes found in yeast also found in aphids? I ask because if not, this could be a stretch for a comparison given the evolutionary distance between the two taxonomic groups. If there are citations for insects for this type of information, they may be added for clarity.

Response 18: Appreciate to point this out. Yes, these genes are highly conserved and are present in yeast. Research on *RIF1* and *BRD8* has primarily focused on their functions, with no reports of their loss in other insect species. The *RIF1* gene has been shown to have a similar function in Drosophila as it does in humans, where it plays a role in regulating DNA replication initiation and maintaining replication fork stability. *BRD8* is involved in DNA repair-dependent chromatin remodeling and the negative regulation of gene expression and is a component of the NuA4 histone acetyltransferase complex. Although the loss of *RIF1* and *BRD8* has not been extensively studied in other insects, the essential roles of these genes in DNA replication, repair, and genome stability are widely recognized.

DMC1 is a meiosis-specific double-strand break repair gene that is associated with the origins of meiosis in eukaryotes. The loss of this gene has been reported in several species, including *Drosophila*, *Anopheles*, *Daphnia pulex*, and nematodes. Investigating the loss of *DMC1* in eukaryotes will help to elucidate the evolution of homologous recombination mechanisms in meiosis and possibly in cyclic parthenogenesis, which warrants further study. In the gene loss section of our manuscript, we have added the following statement (line 296-300):

'The loss of this gene has been reported in species such as *Drosophila*, *Anopheles*,

Caenorhabditis elegans , and *Daphnia pulex*, which exhibits cyclical parthenogenesis. However, the precise effects of *DMC1* loss remain unclear. Investigating the loss of *DMC1* in eukaryotes will contribute to understanding the evolution of homologous recombination mechanisms in meiosis and potentially in cyclical parthenogenesis, which warrants further investigation.'

Figures

Comment 19: Figure 2a: in the methods you stated that you used the adelgid and phylloxerid in the synteny analyses, but they are not in your figure. Where are they and why?

Response 19: The synteny maps for adelgid and phylloxerid are shown in Supplementary Figure 4. In addition to the chromosomal synteny of aphids, we also analyzed three species pairs from the order Hemiptera, including Phylloxeroidea, Reduvioidea, and Fulgoroidea. Our findings indicate that, when considering both inter- and intra-chromosomal rearrangements, the increase in chromosomal rearrangement rates observed in aphids is also present in Phylloxeroidea. However, as this is not the primary focus of our study, it has been included in the supplementary figures.

Comment 20: Figure 2b: There are not taxonomic families called Hormaphididae, Eriosomatidae, etc.

Response 20: We have changed them to Hormaphidinae and Eriosomatinae.

Reviewer #2 (Remarks to the Author):

Comment 1: This manuscript performed a serial analysis and tried to understand the evolutionary patterns of aphid chromosomes. Evolution of aphid chromosomes, indeed, is an intriguing question in the field. This manuscript made efforts to step forward to peer into the question.

I noticed that the authors tried to move beyond stories of the green-peach aphid and pea aphid (ref 9 and 45). However, the logic of this story is pretty weak. The main issue is that the hypothesis is not clear. There is no obvious conclusion generated by the comparative analysis. The manuscript is written descriptively. The story is mixed with the genome sequence of *S. heraclei* and an overview of aphid chromosome evolution. It is very difficult to understand the logic.

Response 1: We sincerely appreciate your valuable comments and acknowledge the concern regarding the clarity of the scientific question and the overall logic of the manuscript. We have made significant revisions to address these points.

In response to your comment, we have explicitly stated the primary scientific question in the introduction (line 71-74). The revised introduction now clearly frames our research focus as the mechanisms and pattern behind chromosomal rearrangement in aphids, investigate whether different aphid lineages exhibit varying rates of chromosomal rearrangement and the reasons behind these differences, and to determine whether a higher rearrangement rate might lead to an increased speciation rate. We believe this clarification better sets the stage for the comparative analysis that follows.

We recognize the need for a clearer narrative between the genome sequence analysis of *S. heraclei* and the broader discussion of aphid chromosome evolution. To address this, we have expanded the analysis of the detoxification gene families's evolution in *S. heraclei*, as this is a key aspect of the manuscript. A new section have been added to the results and discussion part (line 326-363 and 445-462), including the detailed analysis of detoxification gene families and their potential role in adaptive evolution of *S. heraclei*. These additions offer a clearer, more conclusive link between the genomic data and the adaptive strategies of *S. heraclei* in relation to its host plants. The details are as follows in the response below. Additionally, the results section has been reorganized, we have moved the section on 'gene expression patterns in *Semiaphis heraclei*' to the Supplementary Material, further distinguishing the personalized analysis of *Semiaphis heraclei* from the content on aphid chromosome evolution.

We believe these revisions address the concerns raised and significantly enhance the clarity and impact of the manuscript. Thank you again for your insightful feedback.

Comment 2: In the Introduction, they mentioned "incorporate more chromosomal-level aphid genomes", then they sequenced the genome of *S. heraclei*. Actually, many chromosomal-level aphid genomes have already been published, such as Mathers et al. 2022 (Reference 32). With those high-quality genomes, I believe that comparative genomic analysis can be achieved. So, the reason for sequencing *S. heraclei* is very weak. The uniqueness of *S. heraclei* is not addressed enough in the manuscript. *S. heraclei* is a notorious pest; its high-quality genome will be an important resource to understand this pest. In my opinion, the writing will be much better if it is focused on the biology

of S.heraclei.

Response 2: Thank you for your valuable comments. Mathers et al. (2022) published 18 aphid genomes, the majority of these belong to the largest tribe Macrosiphini. Among them, eight genomes were assembled at the chromosome level, but most were generated using short-read sequencing, and several species had previously published genomes. In this manuscript we intend to have broad view of the chromosome rearrangements of aphid, for which we selected the reversative chromosome-level aphid genomes in our manuscript covering the major branches of aphid, including the largest aphid tribe, Macrosiphini, that allow us to generate the reliable results for aphid chromosome evolution. For this purpose, *Aphis fabae* and *Rhopalosiphum padi*, were included in our analysis. The several more chromosome-level aphid genomes have been published since 2024 after we started this project and finished the comparative genomic analysis, that is the main reason that the newly published valuable genomes data were not included in this manuscript. According to the major point in our current work are attempting to have a broad view of the chromosome rearrangements, we selected the available qualified genomes data at the beginning time of this work with the representative sampling of tribe level taxon, which can contribute to the understanding of the chromosomal rearrangement in aphids.

We sincerely thank the reviewer point out the uniqueness of *Semiaphis heraclei* was not adequately addressed in our manuscript, which is also raised be the reviewer 1, and the rationale for sequencing its genome was relatively weak. To address this, we have added content focusing on the host adaptation and evolutionary biology of *S. heracleid* (line 326-363 and 445-462). One of the most distinctive features of *S. heraclei* is its primary host plants, which belong to the genus *Lonicera* (e.g., honeysuckle), and its secondary host plants, which are primarily from the Apiaceae family (e.g., celery, fennel, and Chinese angelica). A significant portion of these host plants are medicinal, producing various secondary metabolites to defend against aphid infestation. Detoxification gene families play a crucial role in the adaptation of insects to host plant secondary metabolites. These include cytochrome *P450* monooxygenases (*P450s*), carboxyl/cholinesterases (*CCEs*), glutathione S-transferases (*GSTs*), and UDP-glucosyltransferases (*UGTs*). Through comparative analysis of these detoxification gene families across multiple aphid species, we revealed that specific expansions and sequence mutations in these genes may contribute to the host adaptation of *S. heraclei*.

Furthermore, *S. heraclei* is a notorious pest that infests medicinal plants, making its genome an invaluable resource for understanding the interaction between aphids and medicinal plants. Our analysis not only highlights the unique evolutionary adaptations of *S. heraclei* but also provides a foundation for future studies on the ecological and evolutionary significance of this species.

Nevertheless, more work and data are needed to further understand the evolution of aphid chromosomes. In future studies, broader sampling and improved sequencing and assembly quality will be essential to better comprehend this issue. We hope these additions strengthen the manuscript and better address the biological significance of *S. heraclei*. Thank you again for your insightful feedback.

Minor:

Comment 3: page 2, line 46, ref 9 is cited inappropriately.

Response 3: Thank you for pointing out the inappropriate citation of reference 9. We have reviewed the content of reference 9 and found that its abstract clearly states, 'The exceptional rate of structural

evolution of aphid autosomes renders them an important emerging model system for studying the role of large-scale genome rearrangements in evolution.' This indeed supports our statement. Therefore, we have retained this citation and added additional references to further support our statement.

Comment 4: Page 2 line 47 "…. 23 subfamilies" lack a reference.
Response 4: Thank you for pointing out the issue of the missing reference for '…. 23 subfamilies'. We have combined this sentence with the next sentence and used the same reference 10 to support them (line 56-57). This not only addresses the issue you pointed out but also makes the text more concise and coherent.

Comment 5: The Introduction should include "Chromosome rearrangement and TE". Ref 70 is a very good paper for TE and gene family evolution.
Response 5: Thank you for your insightful comments and suggestions. We appreciate your recommendation to include the role of transposable elements (TEs) and chromosome rearrangements in the introduction. We have revised the introduction to better integrate this concept and reference the relevant paper (line 50-53).

In the revised introduction, we have explicitly highlighted the increasing recognition of TEs as contributors to genomic rearrangements. We have also incorporated a specific example from Ref 70, demonstrating the significant enrichment of TEs at gene loci related to xenobiotic resistance in aphids, such as cytochrome P450 genes. This addition underscores the potential of TEs to drive adaptive changes in genomic structure and function.

We believe this revision addresses your suggestion effectively and enhances the clarity and relevance of our introduction.

Comment 6: Page 5, line 123, "no inter-autosomal rearrangements". "no obvious inter-autosomal rearrangements were detected" may be better.
Response 6: Thank you for your suggestion. We have changed it in the revised manuscript (line 149).

Comment 7: "Chromosomal rearrangement rates", what it is and how it is calculated?
Response 7: Thank you for inquiring about the definition and calculation method of 'chromosomal rearrangement rates'. 'Chromosomal rearrangement rates' refer to the number or frequency of chromosomal rearrangement events occurring within a specific time period. Typically, this can be calculated by comparing the genomic structures of different species or at different time points. Specifically, it can be calculated through the following steps:

1、 Compare the structures of two genomes and identify all chromosomal rearrangement events (such as inversions, translocations, duplications, etc.). Here, we used MCSCANX to detect genomic rearrangement events.
2、 Divide the number of events by the divergence time of the two species.
3、 Since aphid genomes vary greatly in size, to reduce the impact of genome size on the calculation of chromosomal rearrangement rates, we also divided the result of the previous step by the average length of collinear alignment between the two species.

Reviewer #2 (Remarks to the Author):

Comment1: Abstract. The coherence in the abstract can be improved. The sentences from 29-32, need to be adjusted in order to fit with the context.

Response1: Thank you for your insightful comments. We have carefully revised the abstract to improve logical coherence. Specifically, we have:

1. Added explicit transition to distinguish between two independent findings (chromosomal evolution mechanisms and detoxification adaptation)
2. Restructured the sentence about transposable elements and gene loss/duplication to emphasize their direct relationship with chromosomal evolution
3. Add the content of detoxification gene adaption in the last paragraph to enhance the logical connection between the sentences.

These modifications better clarify the distinct nature of these two important findings while maintaining the overall flow of the abstract. Please see lines 29-33 in the revised version.

Comment2: Please check the tense. When you describe the results, it is better in the past tense. for example, line 151 “we speculate” should be “we speculated”, line 152 “…is mainly,” should be “was mainly”. Line 156 “are generally”, should be “were generally”, etc. Please check the entire paper, which is scientifically amazing now.

Respones2: Thank you very much for your insightful feedback regarding the use of tense in our manuscript. We have carefully reviewed the results section and made the necessary adjustments to ensure that past tense is used consistently when describing our results, as per your suggestion.

For example, as you pointed out, we have changed "we speculate" to "we speculated" (line 151), "is mainly" to "was mainly" (line 152), and "are generally" to "were generally" (line 156). These and other similar changes have been made throughout the manuscript, and we have highlighted all the revised sections in red for your convenience and to facilitate your review.

We greatly appreciate your attention to this detail, and we believe that these changes have significantly improved the clarity and consistency of our manuscript. Thank you once again for your valuable comment, and we hope that the revised version meets your expectations.

Comment3: Fig 2a, are there two X chromosome for E.lanigerum and three for S.chinensis? It looks very weird.

Respones3: Thank you for your question regarding the X chromosome numbers in *E. lanigerum* and *S. chinensis*. You are correct in observing that *E. lanigerum* has two X chromosomes, while *S. chinensis* has three. This phenomenon is indeed unusual, but it is not without precedent in aphids. The occurrence of multiple X chromosomes is known in certain aphid species, particularly within the families *Phylloxeridae*, *Eriosomatinae*, *Lachninae*, and *Drepanosiphinae*. (in paper: General trends of chromosomal evolution in Aphidococca)

We hope this explanation clarifies your concern, and we appreciate your attention to this interesting aspect of our study.