Thesis Evaluation Report

Thesis Title: *Evolutionary Dynamics and Structural Variability of Karyotypes and Sex Chromosomes in Lacertid Lizards in the Context of Other Lacertoideans*
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Summary

This thesis investigates the evolution of sex determination systems and chromosome structure in lizards of the superfamily Lacertoidea, with a focus on the family Lacertidae, known for the stability of its acrocentric karyotypes and its ZZ/ZW sex determination system. This research addresses several unknowns about the evolutionary rate of chromosome number, the variability of the W and Z sex chromosomes, and the putative transition to environmental sex determination (ESD) in certain Lacertidae.

Using bioinformatics, cytogenetic and molecular methods, the author analyses karyotypes and sex chromosomes in several species, revealing several key findings:

Evolution rate of chromosome number: Lacertids show a significantly lower evolutionary rate of change in chromosome number than other lineages of Lacertoidea, suggesting strong conservation of their chromosome structure.

Variability on the W chromosome: High variability in the accumulation of microsatellites and repetitive motifs on the W chromosome is observed, suggesting that although karyotypes are stable, the W chromosome is evolutionarily dynamic.

Stability of the ZZ/ZW system: Analyses confirm the homology of differentiated ZZ/ZW sex chromosomes in all species studied, challenging the hypothesis of transitions to environmental sex determination and underlining the stability of the sex chromosomes in these species.

These findings contribute to the understanding of the evolution of karyotypes and sex determination systems in Lacertoidea, and suggest that the stability of karyotypes in Lacertidae is unique among reptiles and probably related to specific features of their chromosomal evolution. The research suggests future studies of centromeric content in Lacertidae chromosomes to further investigate the reasons for this stability.

Conclusion: The thesis is relevant, innovative and makes a significant contribution to the field of evolutionary genetics. The methodology is sound and appropriate to the stated aims, and the work is well written and structured. I recommend this thesis for approval as it meets high standards of academic research and adds valuable insights to the understanding of the evolution of sex determination systems in vertebrates.

Evaluation

Clarity and Structure

The thesis is well structured with a logical flow that facilitates the reader's understanding of the topic and the findings. The research objectives are clear and well-defined and reflect significant academic relevance, particularly in the field of evolutionary genetics and herpetology. The organisation into clearly defined chapters make for a coherent and smooth

reading experience. The writing is precise, with appropriate use of technical terms. Although the content is dense and specialised, the clarity of the presentation of results and discussion facilitates interpretation of the data, even for readers who are not experts in the field.

Methodology

The methodological approach is well justified, with a clear fit between the methods chosen and the stated objectives. The author uses a robust combination of bioinformatic, cytogenetic and molecular approaches to comprehensively study chromosome evolution in several species of Lacertoidea. The experimental design, based on maximum likelihood models for chromosome evolutionary rates and chromosome synteny comparisons, is appropriate to achieve the stated aims, and the methodology is competently and carefully executed. The use of techniques such as FISH (fluorescence in situ hybridisation), C-banding and microsatellite analysis is appropriate and meticulous, allowing in-depth investigation of chromosomal variability. Finally, the test of homology based on quantitative real-time PCR (qPCR) provides an efficient methodology to search for sex-linked regions.

Contribution to knowledge

The results of this thesis make an important contribution to the knowledge of chromosomal evolution in vertebrates, particularly in lizards. The discovery of a low rate of chromosome number change and the identification of specific patterns in the accumulation of repetitive motifs on the W chromosome reinforce the idea that lizards exhibit a unique evolutionary stability in their karyotypes. Furthermore, the thesis challenges previous hypotheses on the existence of environmental sex determination in lizards, providing evidence against this theory and suggesting a high conservation of the ZZ/ZW system.

Relevance and originality

This thesis addresses a highly relevant topic in evolutionary genetics, focusing on the evolution of sex determination systems and chromosomal structure in Lacertidae lizards. Known for their remarkable chromosomal stability, Lacertidae represent an evolutionary exception among reptiles. This work provides a unique perspective on how genetic mechanisms and natural selection contribute to chromosomal conservation and the evolution of sex determination, providing insights that are crucial for understanding karyotype evolution and sex determination diversity in reptiles and vertebrates more broadly.

The thesis is highly original, using an innovative approach to study the variability and stability of sex chromosomes and karyotypes. Using advanced methods - including syntenic analysis of NGS data, cytogenetic and molecular techniques - it addresses previously overlooked aspects such as karyotype evolution, the dynamics of repetitive motifs on W chromosomes and the evolutionary stability of the ZZ/ZW system. By challenging previous assumptions about environmental sex determination in some species, this work makes a unique and valuable contribution to the field and improves our understanding of chromosomal evolution in Lacertidae.

Conclusion and Recommendation

The thesis presents a rigorous, comprehensive and innovative investigation into the genetics and evolution of lacertid lizards, addressing key questions about chromosomal diversity, stability and sex determination in Lacertidae. This well-founded research provides a solid basis

for understanding the mechanisms that support chromosomal conservation and makes valuable contributions to the field of evolutionary biology. With some of the findings already published in peer-reviewed journals, the thesis effectively supports its aims and advances knowledge in this specialised field. I recommend that the thesis be accepted.

Recommendations

- Figures for chapter 1 have no figure name and do not seem to be in the right order.
- A figure with a phylogeny including all the families mentioned in the thesis would be very useful in the introduction for readers not expert in reptiles. Also, the use of common names may difficult the comprehension for those no familiarized with these species.
- Expand the discussion on the implications of findings in the context of the conservation of the studied species.
- It is recommended to provide more details on specific techniques (cytogenetic techniques), as they are not included in the chapters presented.

Evaluator: Mónica Bullejos

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QUESTIONS

1. Is polyploidy only observed in partenogenetic lineages? Why? Any Hypothesis? Any relation to hybridisation?
2. Are the figures for chapter 1 in the right order?
3. Your synteny analysis is restricted to species with assembled genomes. Would it be possible/feasible to extend the analysis to other species using chromosome painting?
4. Why do you use PNA probes for telomeric and microsatellite motifs? Any differences with DNA probes?
5. Why were selected the 4 species included in figure 5 (chapter 2)? Has the W chromosome from *G. parisina* C-positive bands/regions?
6. How do you explain the discrepancy between your results about the Z chromosome conservation and the results from chromosome painting using probes from Z chromosome of *I. monticola* on chromosome from *L. schreiberi*?