Bioinformatic Analysis of Pericentromeric Repeats in Unisexual Lizards *Darevskia* of Hybrid Origin

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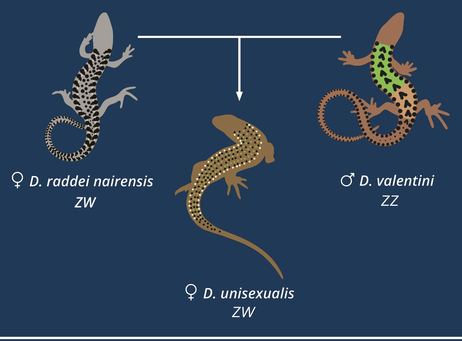
Introduction

Satellite DNA is the main component of the pericentromeric regions of chromosomes and consists of tandem repeats. Even though the genomes of many organisms have already been sequenced and annotated, satellite DNA remains poorly understood in all of them, due to algorithmic challenges presented by the assembly of its sequence [1].

In this work, we study a complex of three lizard species: *Darevskia raddei nairensis*, *D. valentini*, and *D. unisexualis*. The first two species diverged more than 18 million years ago and interbred to form a parthenogenetic species *D. unisexualis* [2]. This hybrid species is of a particular interest because of its true unisexual reproduction with the preservation of a diploid set of chromosomes through generations.



Aims

1. Bioinformatically predict the sequences of species-specific FISH probes to discriminate between the paternal and maternal chromosomes of *D. unisexualis.*
2. Verify the predicted FISH probes on the mitotic metaphase karyotypes of *D. raddei nairensis*, *D. valentini* and *D. unisexualis*.
3. Analyze the hybrid karyotype using designed probes to distinguish between maternal and paternal lineages according to chromosome morphology.

References

[1] Nurk et al., 2022. PMID: 35357919
[2] Spangenberg et al., 2020. PMID: 32457493
[3] Ciobanu et al., 2003. PMID: 14714467

Acknowledgements

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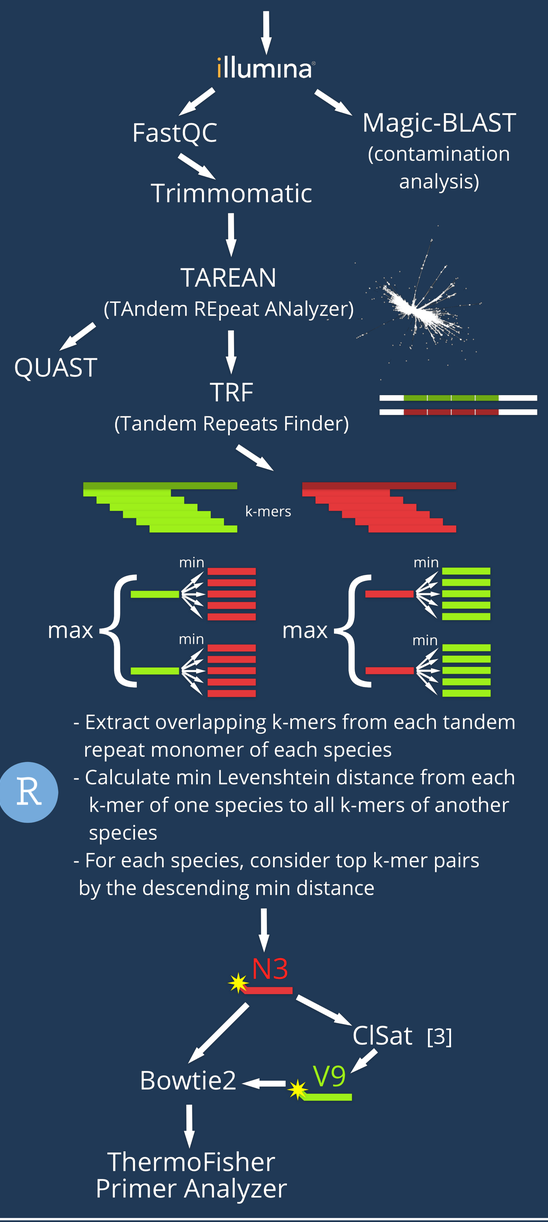
Materials and methods

Microdissection of pericentromeric regions of *D. raddei nairensis* and *D. valentini*



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- Extract overlapping k-mers from each tandem repeat monomer of each species
- Calculate min Levenshtein distance from each k-mer of one species to all k-mers of another species
- For each species, consider top k-mer pairs by the descending min distance

Results

1. Probes **N3** and **V9** differentially highlight *D. unisexualis* chromosomes inherited from parental species.

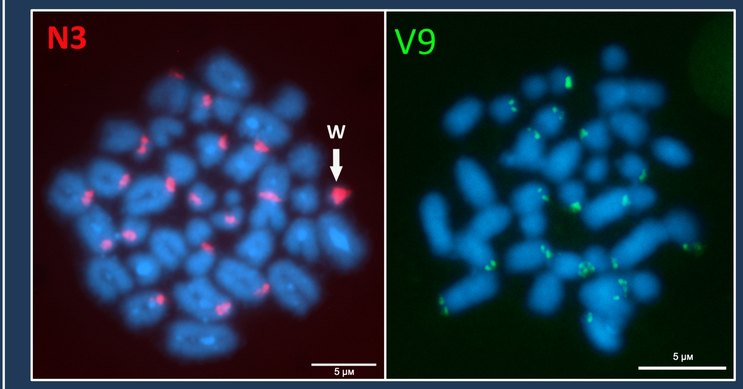

Figure 1. *D. unisexualis*

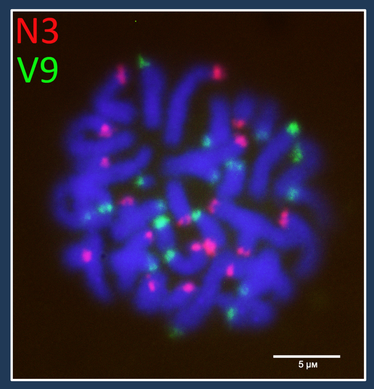

Figure 2. *D. unisexualis*

2. Probes **N3** and **V9** hybridize to all chromosomes in the karyotypes of only the respective parental species, confirming that these probes are species-specific DNA markers.

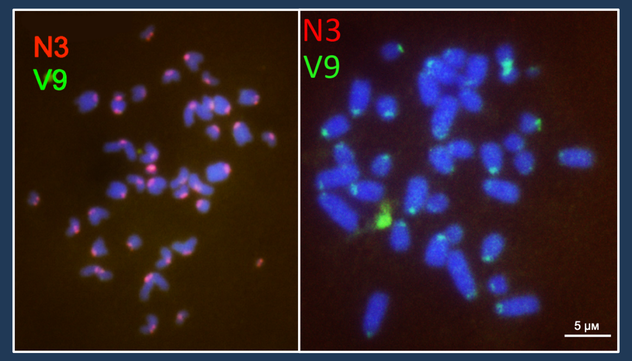

Figure 3. *D. raddei nairensis* (left) and *D. valentini* (right)

3. Probe **N4** demonstrates specific signals in the peritelomeric regions of the W chromosome.

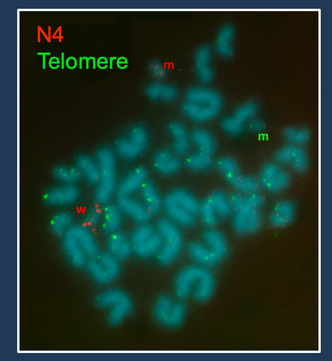

Figure 4. *D. unisexualis*

* N probes (from *D. raddei nairensis*) are coloured in red, V probes (from *D. valentini*) are coloured in green.

Conclusions

1. We bioinformatically predicted species-specific FISH probes for the two parental lizard species, *D. raddei nairensis* and *D. valentini*, and successfully validated the probes on mitotic metaphase plates of the hybrid species *D. unisexualis*.
2. Using the designed probes, we detected the W chromosome (the strongest red signal from the *D. raddei nairensis* probe), thus confirming the maternal species for *D. unisexualis*. We also found an additional peritelomeric W chromosome-specific probe.
3. Finally, our data suggest that the ClSat family of tandem repeats is the most prevalent one in the three *Darevskia* species and that these repeats diverged between *D. raddei nairensis* and *D. valentini* enough to design species-specific DNA markers.