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Is There Sex-Specific DNA In The Tuatara, A Reptile With Temperature-Dependent Sex Determination?

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Abstract

It is widely viewed that there is a dichotomy of sex-determining mechanisms within the reptiles: species either exhibit genotypic sex determination or temperature-dependent sex determination (TSD). However, very few species have been examined for both modes. Although it is often considered that the two mechanisms are mutually exclusive, there is evidence that there may be a weak genetic sex-determining mechanism in species in which the primary sex-determining mode is temperature-dependent sex determination. This infers that some TSD individuals may be sex-reversed; that is, their sexual genotype is discordant with their sexual phenotype. This hypothesis of an underlying genotypic system may also be linked to the question of the evolution of sex-determination within the reptiles. The discovery of sex-specific DNA within a TSD reptile could suggest that genotypic sex determination is ancestral and TSD has evolved many times over within independent reptile lineages.

This study tested the hypothesis that there is a genetic component to sex determination in TSD species. This was accomplished by searching for sex-specific DNA in the tuatara, a reptile with temperature-dependent sex determination, using two different molecular genetic techniques.

The major undertaking of the experimental programme was the completion of a comprehensive minisatellite DNA profiling survey. This incorporated 14 restriction enzymes and five different polycore DNA probes; in total, 66 different probe/enzyme combinations were tested for tuatara genomic DNA. None of the DNA profiles revealed sex-specific fragments. Furthermore, a significant difference in mean fragment numbers for males and females was not detected for any of the probe/enzyme combinations.

In addition, a RAPD analysis was conducted in a search for a molecular sex marker in the tuatara. A total of 27 random-sequence oligonucleotide primers were used to successfully amplify anonymous products from the genomic DNA of male and female tuatara. Again, no sex-specific fragments were detected.

Thus, evidence of sex-specific genetic differences in the tuatara was not found. This result fails to refute the null hypothesis that there are underlying sexual genotypes in the tuatara. This finding may reflect the absence of genetic sex differences in the tuatara. Alternatively, it might also be the result of accidental inclusion of sex-reversed individuals within the analyses, a situation which could have obscured the sex-specific nature of any sex-linked fragments. It would appear that the key to solving the question of sex-specific DNA within TSD reptiles such as the tuatara lies with the problem of ensuring sex-reversed individuals are excluded from molecular analysis.

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Thesis Structure And Format

I have arranged this thesis in the following manner. **Chapter 1** gives a general introduction to the tuatara, describing its evolutionary position, taxonomic status, distribution, and general biology. The aspect of tuatara ecology that is the focus of this study, sex determination, is introduced. Finally, the aim of the thesis is explained, in terms of its relevance to scientific study and also its application to the conservation of this rare species.

In **Chapter 2** I provide background material and theory relevant to this research. The phenomenon of temperature-dependent sex determination (TSD) is described in some detail, including a general review of its occurrence within the four major living groups of reptiles (including the tuatara). The concept of genetic sex differences in a species that exhibits temperature-dependent sex determination is examined. Current insights into the possible molecular mechanisms of TSD are discussed, particularly with respect to reconciling the concept of an underlying genotypic mode of sex determination interacting with the TSD mechanism. This discussion is used to give a context to the methodological approach of the study.

Chapter 3 presents a detailed account of the major experimental undertaking of the investigation; a comprehensive minisatellite DNA profiling survey aimed at testing the hypothesis that tuatara have sex-specific DNA. This survey is divided into three distinct phases, consistent with three different sets of tuatara blood samples. The results of a large number of probe/enzyme combinations are presented and discussed.

Chapter 4 is an account of a brief investigation employing RAPD (Randomly Amplified Polymorphic DNA) assays as a further attempt to detect a molecular marker for gender in this species. In this study, a large number of random-sequence oligonucleotide primers were used to amplify anonymous PCR products from male and female tuatara. Results are presented and discussed.

Chapter 5, the final chapter, presents a summary of the findings of the research. Following a general conclusion, there is a discussion of potential avenues of investigation for future research into the question of sex determination in the tuatara (and TSD reptiles in general).

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