5-1-2018

ANALYZING GENE CONVERSION IN PARTHENOGENS

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by

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Presented to the Faculty of the Honors College of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

HONORS BACHELOR OF SCIENCE IN BIOLOGY

THE UNIVERSITY OF TEXAS AT ARLINGTON

May 2018

ACKNOWLEDGMENTS

This project and the progress made throughout my research would not have been possible without the help of Dr. Matthew Fujita. He granted me the opportunity to work in his genomics lab and for that I am forever indebted to him. His guidance and aid were what pushed me forward and made all this possible. I would like to also thank other members of the lab who aided me when I needed it: Jose Maldonado, Kathleen Currie, and Trung Nguyen.

Furthermore, I'd like to thank my family for giving me the love and support throughout my entire undergraduate experience. My professors deserve credit for giving me the foundation needed to excel in my research.

Lastly, all praise to God, the most beneficent, the most merciful.

May 20, 2018

ABSTRACT

ANALYZING GENE CONVERSION

IN PARTHENOGENS

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The University of Texas at Arlington, 2018

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Parthenogenesis is a type of asexual reproduction in which an unfertilized, typically female, gamete develops into a genetically identical clone of the mother. Gene conversion is the unidirectional transfer of genetic material from one DNA strand to another that is from a donor strand to an acceptor strand; this mechanism is not expected to occur in parthenogens. This project determines whether gene conversion occurs in a few species of parthenogens. The 18S gene was first amplified via PCR and the resulting amplicons were run on a gel to confirm successful amplification before sequencing. We found that 18S was genetically identical in *Aspidoscelis*, however, sequences of *Heteronotia* were seen to not be heterozygous indicating that gene conversion is taking place. Further study on this species is ongoing.

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CHAPTER 1

INTRODUCTION

1.1 Processes that generate genetic variation

Genetic variation is what drives a species to produce offspring that are different. Without genetic variation the catalogue of species that we have would not exist. Genetic variation can be brought about by a couple of known methods, which will be discussed in further detail.

1.1.1 Mutation

Mutations are genetic alterations in the genetic sequence of the individual. These are random alterations and cannot be predicted until they occur. A mutation can affect the traits of the individual, either phenotypic or genotypic traits. Mutations can either be hereditary or acquired. Hereditary mutations are those that are inherited from a parent, while acquired mutations are those that occur during an individual's life, and it can be seen that some mutations occur more frequently than others.

1.1.2 Recombination generates new variation

Recombination is a method that also produces genetic variation within individuals. It occurs during meiosis in eukaryotes and works by exchanging genetic material between homologous chromosomes, which can result in the exchange of genetic variation. In eukaryotes, recombination involves the presence of homologous chromosomes and it is within these homologous chromosomes that genetic sequences can be altered to produce new traits.

1.2 Process that can remove genetic variation–gene conversion

Not all genetic variation is beneficial for the individual. Mutations can cause the individual problems and diseases; sickle cell anemia is an example of a disease that is caused by a mutation. An individual's cells are constantly undergoing genetic replication, consequently there must be a mechanism by which these mutations are kept at bay.

1.2.1 Recombination-based mechanism

In double stranded DNA, there may be situations where one strand is damaged. The recombination-based mechanism repairs damaged single-stranded DNA by attaining the other strand it needs via recombination. Recombination occurs using a template strand and copying it to give the single strand the complementary strand it needs. Recombination does not actually repair the nucleotides that have been damaged but have, in essence, been given spare parts. One byproduct of recombination is gene conversion, in which repairing damaged DNA will cause one allele to be converted to another allele. This process actually removes variation, because a previously heterozygous site will be changed to be homozygous.

1.3 Parthenogenetic lizards

This project used parthenogenetic lizards to try and determine if gene conversion occurs in these reptiles and what mechanism is being used.

1.3.1 What are they?

Parthenogenetic lizards are asexual reptiles. Theses are rare reptiles, which undergo reproduction by asexual means, in which daughters are identical clones to mothers. It was seen that these species of lizards were undergoing mutations. This is not

supposed to be the case if the offspring is supposed to be genetically identical to the parents. (Kearney et. al., 2009)

1.3.2 Hybrids

Genetic hybrids are formed when two species mate and produce an offspring whose genetic identity is a mixture of the two parents. There are many studies that have been conducted in order to determine how these parthenogens arose. Many believe that the process of hybridization caused these parthenogenetic lizards to be produced. These hybrids are the species that were used to conduct the experiment. (Hillis et al., 1991)

1.3.3 Genetic consequences–population that is all female and genetically identical

With asexual populations, the offspring are genetically identical to the parents. Therefore, we can also infer that if a situation arises where one member of the population is negatively affected then it will affect all members of the population. This is why genetic variation is important within a population, as it allows future generations to be better capable at combatting tough situations better than previous generations. My project will investigate processes affecting genetic variation in parthenogenesis lizards. (Hartfield, 2016)

1.4 My project

This section will discuss why I decided to partake in this project and the importance behind the findings. The last paper written on this specific project was written over twenty years ago, so there is a huge possibility given the improved quality of instruments and resources that results of huge importance could be produced.

1.4.1 Using parthenogenesis to investigate genomic mechanisms–gene conversion

The purpose of this project is to determine if gene conversion is taking place in the parthenogens we are examining and if there is evidence of gene conversion. We would then look deeper to determine how this gene conversion is taking place. (Fujita et. al, 2009)

1.4.2 18S and 28S genes–Hillis and Moritz

Hillis and Moritz looked at the 18S ribosomal DNA to determine the mechanism of concerted evolution in unisexual hybrids (parthenogens). Their paper, written in 1991, determined that there were two possibilities why homogeneity exists within these species. The first possibility was unequal crossing over of genes and the second was gene conversion. Using restriction digestions, it was determined that the mechanism for which homogeneity exists is gene conversion due to the fact that the ribosomal DNA is biased towards one of the parental sequences. This finding was more in tune with the gene conversion.

1.4.3 The lizards

I used two lizard systems: whiptails (*Aspidoscelis*) and geckos (*Heteronotia*). For my project, I focused on three species of whiptails (two sexuals: *A. marmorata* and *A. gularis*; one parthenogen: *A. tesselata*). For *Heteronotia*, I focused on two sexual lineages termed “CA6” and “SM6” and one parthenogen termed “3N1”. Because the parthenogens are hybrids, we expect them to be heterozygous. However, if gene conversion occurs, then the parthenogens will be homozygous.

CHAPTER 2

METHODS

2.1 DNA Extraction

We would first need the DNA of the parthenogens whose genes we were sequencing. DNA extraction is the process, which pulls DNA out of the cells of these species. There are several different methods used to extract DNA from its cells, but because these cells have similar anatomical features most methods have similar steps used for extraction. (Elkins, 2013) The process by which DNA is extracted usually follows these simple steps:

1. The cells must be lysed to expose its constituents
2. The DNA must be separated from the constituents of the cell
3. The DNA is then isolated

2.2 DNA quantification/running DNA on a gel

The extracted DNA must be examined and tested to see if there are any contaminants or unwanted DNA fragments present. Without this process, the DNA that is sequenced could be contaminated DNA, therefore, making the experimental results useless. In order to do this, DNA are run on agarose gel. DNA samples, along with a negative control, are run on the gel to determine whether contaminants are present. On the gel we should have no band along the well containing the negative control and thick clear bands along the wells containing DNA samples. Each well must only produce one band otherwise there might be contamination.

2.3 PCR (Polymerase Chain Reaction)

PCR is used to amplify the DNA samples we have obtained. This is because, for most studies of genetic material, significant amounts of the DNA sample is needed to achieve conclusive results. The steps of PCR are as follows:

1. The template DNA is dissociated at 95 degrees Celsius
2. The DNA is then cooled so the primers can anneal to it
3. Taq DNA polymerase extends the primers

These steps constitute one PCR cycle. The number of DNA strands produced corresponds to the equation 2^n where n is the number of cycles.

2.4 Cleaning up the PCR product

After the PCR cycles have been completed, the DNA needs to be extracted and isolated so that it may be sequenced. This is done using magnetic beads. Because DNA is negatively charged, it is possible to extract the amplified products from the solutions they are in. Magnetic beads and the DNA bind together, and once this happens the solutions are then placed in magnets. The supernatant is removed leaving DNA and the magnetic beads.

2.5 Sequencing

Now that the DNA has been isolated, we need to prepare it to be sequenced. We use the primer initially used during PCR and Formamide to prepare the DNA to be sequenced. Using these along with 5x buffer, the samples are then returned to the thermocycler, and the Big Dye protocol was applied to it.

2.6 Cleaning the sequencing product

Once the sequencing reaction is complete, the solution needs to go to through one last cleanup before it is ready to be sequenced. This reaction is called the Ethanol Precipitation of Sequencing Products. Using Sequencing Clean-up solution and different concentrations of ethanol, as well as centrifuging, the reaction was used to produce solutions that were used by the genomics lab to produce sequences of the DNA we needed.

2.7 Visualizing sequences on the computer

Each DNA sample had a pair of genes that were sequenced. A forward gene and a reverse gene, these DNA strands are to be analyzed on a software known as Geneious, where each DNA strand can be seen in great detail going down to the specific base pairs of which it is composed

2.8 Alignment

Each sample produced two DNA strands. The software allows the strands to be aligned together so that the base pairs can be compared. The software allows us to see a conclusive picture of the genetic sequence of each sample.

2.9 Looking for variants

Comparing both sequences, we can determine whether or not there is genetic variation. If the base pairs are different, we will know that there has been some mutation that has occurred.

CHAPTER 3

RESULTS

3.1 Results of PCR

After the amplification of the DNA is performed by PCR, the product was run on gels to ensure contamination had not occurred. Each set of samples underwent multiple PCR runs, and below are attached figures of the samples that were run that were further sequenced.

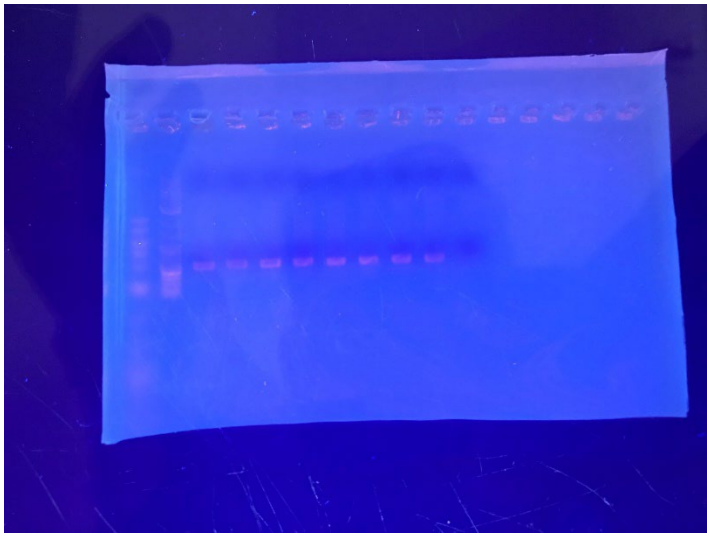

Figure 3.1: Gel of PCR product containing *Aspidoscelis gularis* and *Aspidoscelis tesselatata*

Table 3.1: Well description of the gel in Figure 3.1

Well Number	2	3	4	5	6	7	8	9	10	11
Constituent	Ladder dye	*Aspidoscelis* gularis				*Aspidoscelis* tesselata				Negative Control
Species Label		KLC 029	KLC 030	KLC 034	KLC 035	KLC 007	KLC 066	KLC 011	KLC 012	

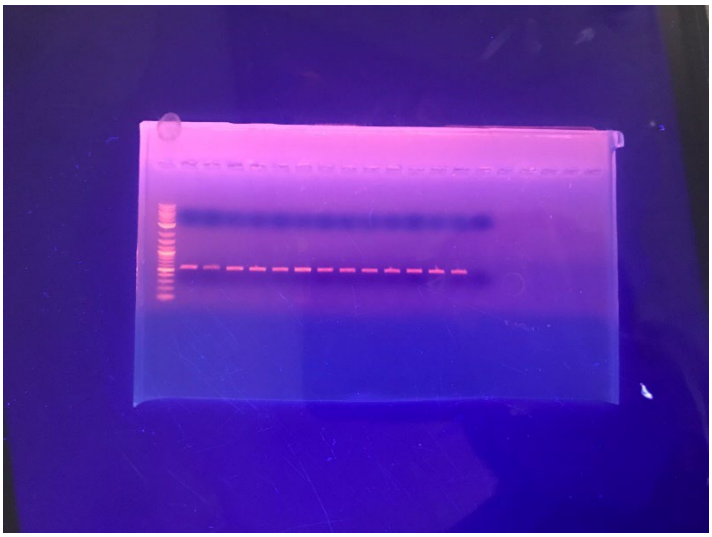

Figure 3.2: Gel of PCR product containing *Heteronotia*

Table 3.2: Well description of the gel in Figure 3.2

Well Number	1	2	3	4	5	6	7
Species Label	MKF 689	MKF 690	MKF 707	MKF 708	MKF 709	MKF 718	MKF 730
Well Number	8	9	10	11	12	13	14
Species Label	MKF 731	MKF 737	MKF 740	MKF 742	MKF 751	MKF 756	Negative Control

3.2 Chromatogram and alignment

The sequenced DNA was run on Geneious and the following chromatogram and alignment was produced.

Figure 3.3: Chromatogram of *Heteronotia*

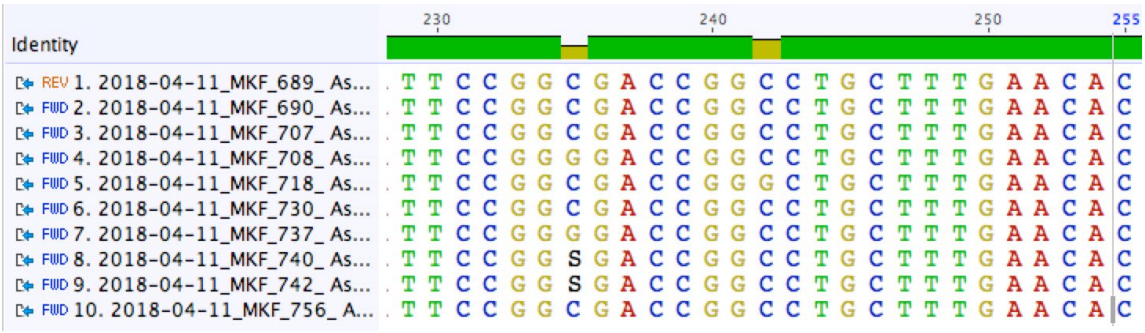

Figure 3.4: Alignment *Heteronotia* sequences

CHAPTER 4

DISCUSSION

4.1 Whiptails–no genetic variation in 18S

The first genus of parthenogens we analyzed was *Aspidoscelis* and, the species we examined were *gularis* and *tesselata*. We were looking to see whether any genetic mutation had occurred between the two species. Looking at the data obtained after sequencing, all the samples possessed identical genetic sequences showing that there had not been any genetic mutations between them. However, this is not to say that gene conversion is not a mechanism by which this genus mutates. Further study using different species of the same genus will give us a better understanding as to whether gene conversion is taking place.

4.2 *Heternotia*–on going

Time being a constraint, we were only able to conduct one round of sequencing of the *Heteronotia* samples. When we processed the sequences and aligned them, we found some variation among the samples. While our small sample size prohibits us from a comprehensive analysis, we have found possible evidence of gene conversion. For instance, we expect that the parthenogen samples to be heterozygous as seen with sample MKF 740 and MKF 742. However, all other samples of *Heteronotia* were seen to be homozygous, indicating that gene conversion has happened.

4.3 Future directions

Our results with *Heteronotia* indicate that gene conversion is likely happening, confirming the study by Hillis and Moritz (1991). However, our sample size was very limited, and we must expand our sampling to include more parthenogens and more sexuals to verify that gene conversion is in fact happening. This evidence suggests that gene conversion is eliminating genetic variation from parthenogenetic lineages (converting heterozygous sites to be homozygous). We are also continuing our investigation by sequencing additional rDNA genes that may harbor more variation.

APPENDIX A

GLOSSARY

Amplicon: An amplicon is a piece of DNA or RNA that is the source and or product of amplification.

Asexual: Not involving the fusion of gametes.

DNA: Deoxyribonucleic acid, the foundation that encodes information for living creatures

Eukaryote: An organism consisting of a cell or cells in which the genetic material is DNA in the form of chromosomes contained within a distinct nucleus.

Gamete: A mature haploid male or female germ cell that is able to unite with another of the opposite sex in sexual reproduction to form a zygote.

Gene: A distinct sequence of nucleotides forming part of a chromosome.

Homologous Chromosome: A pair of chromosomes that match up at meiosis and are identical in morphology and arrangement.

Meiosis: A type of cell division that results in four daughter cells each with half the number of chromosomes of the parent cell.

Sickle Cell Anemia: A hereditary blood disorder resulting in anemia due to a mutation in the allele coding for the beta chain of hemoglobin.

REFERENCES

Elkins, K. M. (2013). DNA Extraction – An Overview. Retrieved from ScienceDirect Topics

Fujita, M. & Moritz, C. (2009). Origin and Evolution of Parthenogenetic. Cytogenetic and Genome Research, 261-272

Hartfield, M (2016, July). On the Origin of Asexual Species by Means of Hybridization and Drift Retrieved from Molecular Ecology, U.S. National Library of Medicine

Hillis, D. M., Moritz, C., Porter, C. A., & Baker, R. J (1991). Evidence for Biased Gene Conversion in Concerted Evolution of Ribosomal DNA. Science, 308-310.

Kearney, M., Fujita, M. K., & Ridenour, J. (2009). Lost Sex in the Reptiles: Constraints and Correlations. Victoria: Department of Zoology, The University of Melbourne

BIOGRAPHICAL INFORMATION

Murtaza Mucklai was born in Karachi, Pakistan. After completing his Cambridge International A Levels, he moved to Arlington, Texas to pursue his Honors Bachelors of Science in Biology and Chemistry. Two years of research under Dr.Matthew Fujita gave him a greater understanding of the scientific process and the research that goes into every scientific finding. After graduating, Murtaza plans to work as a scribe and finish his application for medical school. His end goal is to become a licensed physician.