DNA Barcoding of Caffeine Containing Plants

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Introduction

Caffeine is a central nervous system (CNS) stimulant of the methylxanthine class. It is the world's most widely consumed psychoactive drug. According to the Mayo Clinic, most adults can safely consume 200-300 mg of caffeine per day. Coffee is as important to everyday early risers as it is to the global economy.The International Coffee Organization, more than 8.7 million tons of coffee were produced in 2013 and sector employed nearly 26 million people in 52 countries during the year of 2010. By looking at the evolution of caffeine, we can have a broader prospect of the chemical we consume everyday and it may also provide insights to coffee and its substitutes producers.

Caffeine is occurring in 60 plants naturally; some plants with high caffeine contents are *Camellia sinensis*, Khat, Cassina, Kola nut, and Cocoa. It then came to our mind that are there any evolutionary relationships between plants that produce caffeine? If so, what are the relationships. If not, what are the possible ways to produce caffeine? To find the answer, we started a project based on DNA Barcoding.

Materials & Methods

First, we collected 12 samples and used the silica method to extract their DNA.
We followed the instructions and carried out the extractions. Then, we used Polymerase Chain Reaction (PCR) to amplify the 12 DNA samples. We separated the amplified DNA on the gel. After 30 minutes of electrophoresis, we took pictures of the gel under UV light to get the result of DNA bands on the gel. On the first try, 2 out of 12 of our samples worked. We sent these two samples, FTT006 and FTT008, for sequencing.

On the second try, we used another method to extract DNA from the remaining samples, using the Qiagen Dneasy Mini Plant Kit. We repeated the PCR and gel electrophoresis as above and got the picture of gel. This time 4 out of 9 of our samples worked:sample 7, sample 9, sample 11, and sample 12. We sent their DNA for sequencing. The other samples did not work well on PCR.

Data Analysis

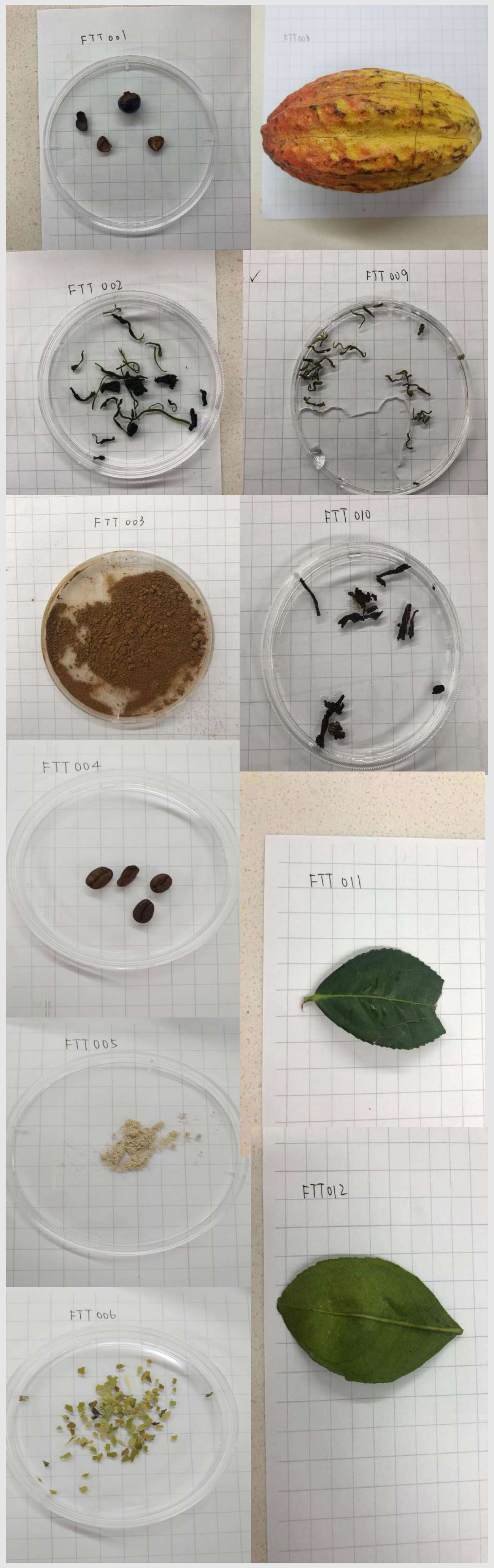

Figure 1 Samples

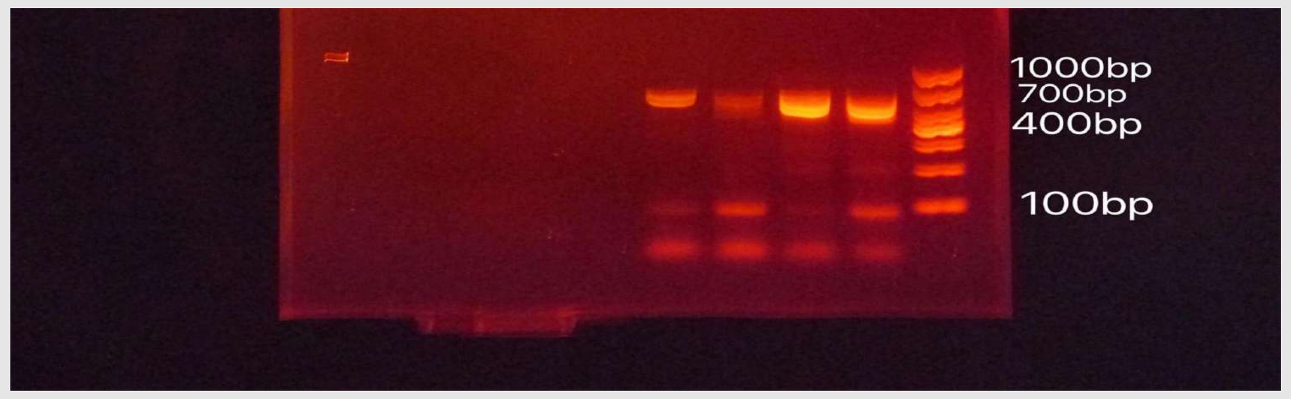

Figure 2 Gel picture FTT008A FTT008B FTT008C FTT008D

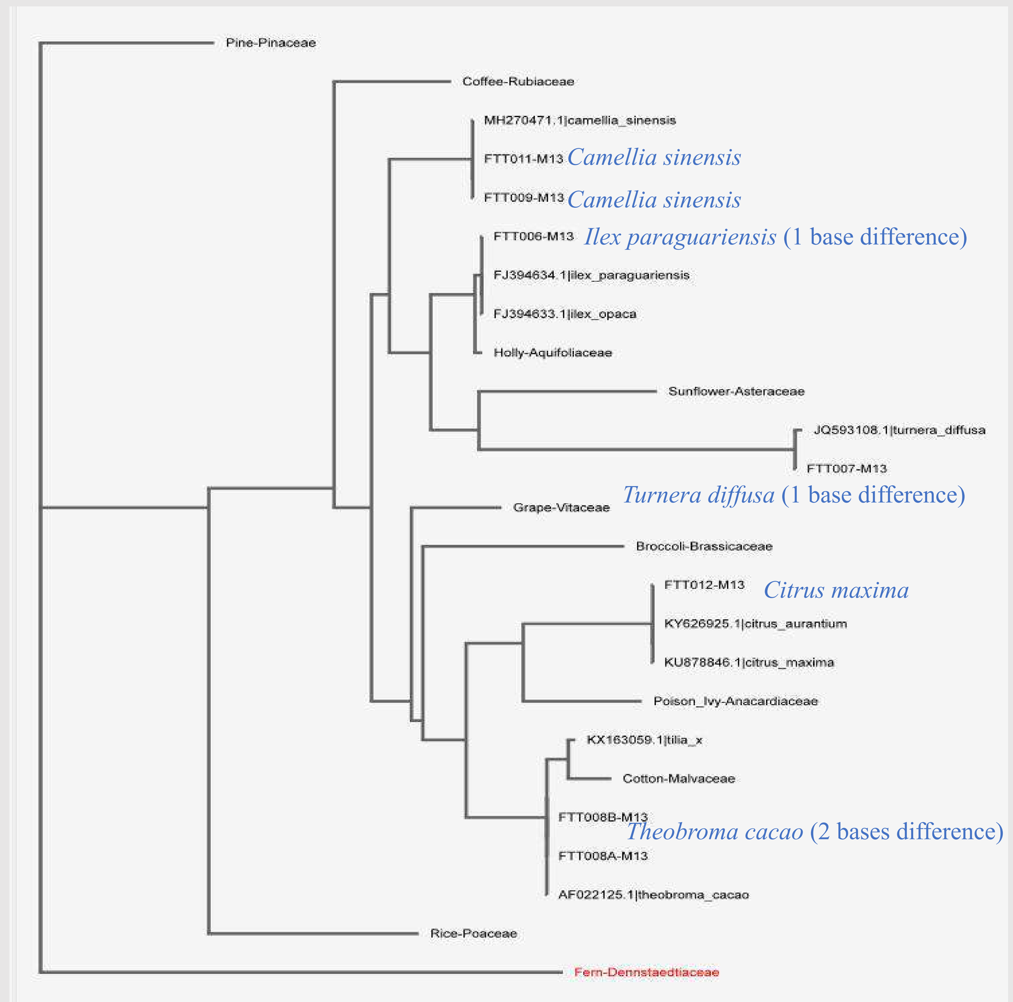

Figure 3 The PHYLIP ML tree Diagram

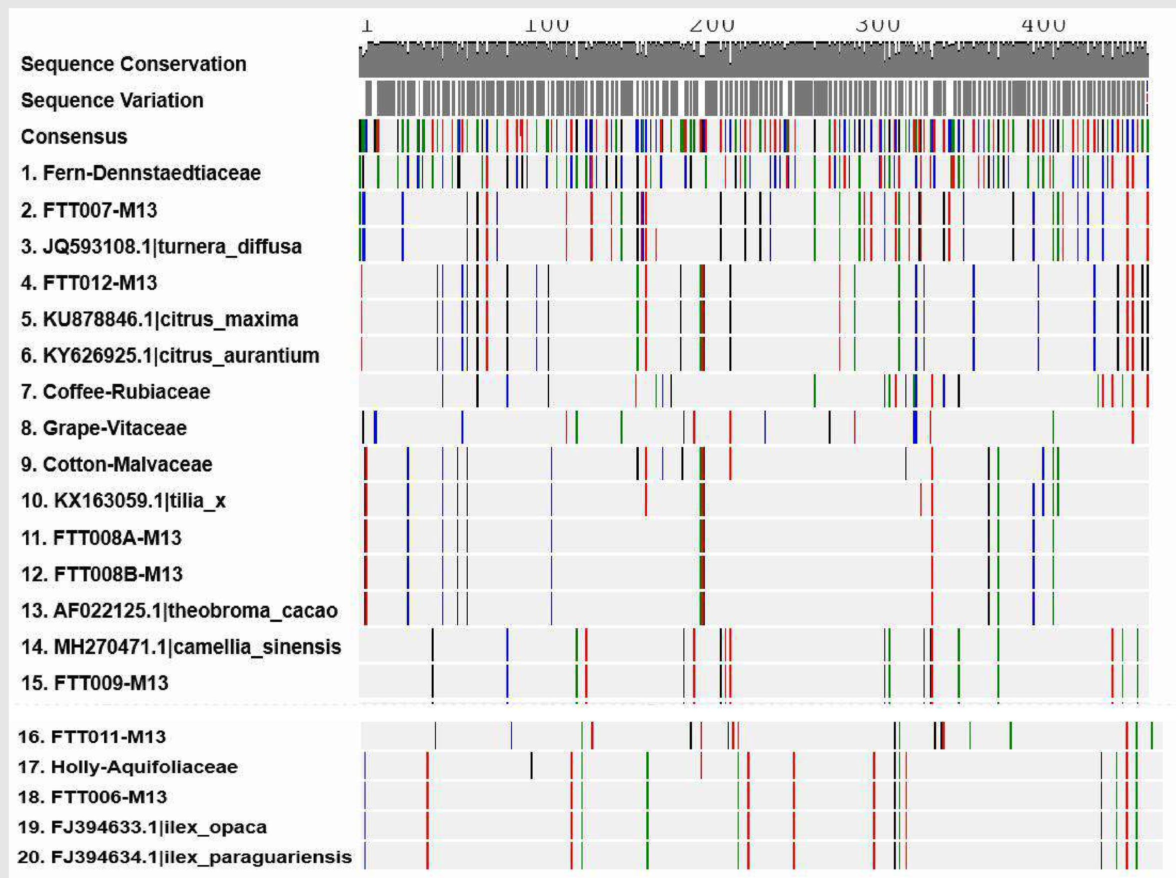

Figure 4 DNA Sequence Aligment for the Barcoding Region

The PHYLIP ML tree constructed from our samples shows caffeine-producing plants evolved independently. For example, the *Camellia sinensis*(tea) is very distant from the *Theobroma cacao* (cocoa tree) on the tree. These two species both contain caffeine, but they share no recent common ancestor. The cocoa is more closely related to Broccoli than to tea or other plants that produce caffeine. Other samples show similar pattern as the one discussed above, so we know these caffeine-producing plants all evolved independently.

Discussion

The evolution which is responsible for generating similar traits in unrelated organisms is called convergent evolution. In plants, one of the most prominent examples of convergence is that of caffeine production, which has independently evolved in numerous species.

According to scientists at Western Michigan University, caffeine-producing plants have taken a number of different biochemical routes to synthesize the stimulant. Studies have shown that coffee (*Coffea arabica*) and tea (*Camellia sinensis*) use different enzymes to generate caffeine—xanthine methyltransferases (XMT) and caffeine synthases (CS), respectively. Comparisons of the coffee genome (*Coffea canephora*) to genes from cocoa (*Theobroma cacao*) and tea have provided genetic confirmation of the convergent rather than divergent evolution of caffeine production. Barkman and his colleagues have compared the covergent evolution process of tea and coffee to three additional species: cocoa, guarana(*Paullinia cupana*), and orange (*Citrus sinensis*).

Despite utilizing different enzymes, coffee and tea plants make caffeine via the same biochemical steps: methylation of the nucleoside xanthosine, followed by two further methylation steps to make caffeine. However, the other plants studied make use of a similar molecule, called xanthine. Some or all of the intermediate molecules in the pathways also differed in cocoa, orange, and guaraná plant caffeine synthesis. The research team have found that the earliest synthetic ancestor, which the Citrus lineage would have shared with coffee, could be methylate benzoic acid and salicylic acid (carboxylic acids involved in floral scent, pathogen defense, and more), but could not be methylate xanthine or xanthosine.

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