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From The Fields to the Molecules: An Investigation of Mitochondrial DNA Inheritance in Strawberries

Monica Quimby
University of New Hampshire

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From The Fields to the Molecules: An Investigation of Mitochondrial DNA Inheritance in Strawberries

—Monica Quimby (Edited by Brigid C. Casellini)

My first job was picking strawberries for 50 cents a quart in the summer of 2000. I loved the smell of ripened fruit and the experience of being embedded between the rows of strawberry plants. I worked in the strawberry fields during high school, and when I came to UNH I started taking care of strawberry plants in the greenhouses. I remember being tested by Dr. Tom Davis: "What is the difference between these two strawberry plants?" he asked. Then I carefully listed all of the physical differences from the veins on the leaves, to the shape and color on the fruit. I had a deep understanding of the strawberry plant at a horticultural and physical standpoint, but I wanted to know more about the inheritance of genes and traits.

Figure 1: The author in Dr. Davis's lab at UNH.

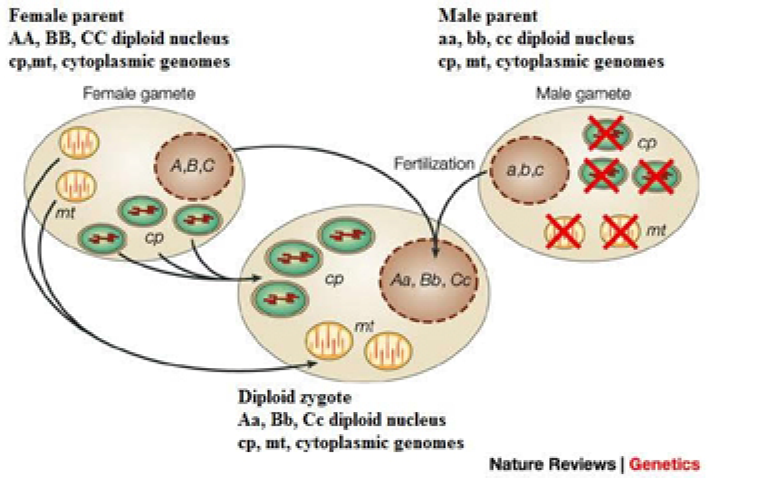
In 2009, I received an academic grant from the Undergraduate Research Opportunities Program (UROP) to help explore the evolutionary path leading to the cultivated strawberry. DNA is a very important tool that helps us understand ancestry and inheritance. My goal was to test the hypothesis that mitochondrial DNA (mtDNA) is maternally transmitted in strawberry. The importance of these findings would be threefold. First, for biologists and the science of agriculture, more information on the strawberry genome and its ancestry will allow for informative comparisons with other closely related species, such as apples, cherries, and peaches, all of which are part of the Rosaceae family (Davis 2006). This could in turn help to define the common ancestor of the Rosaceae species.

Second, determining the mitochondrial mode of transmission can tell us several important things about a plant, such as its cross-hybridization compatibility, its capacity to transmit certain forms of disease resistance, and whether male sterility exists in the cytoplasm (Vaillancourt *et al.,* 2004, Quenzar *et al.,* 2001). Third, the type of mitochondrial transmission affects the fitness and adaptability of the plants, and in some instances plant/pest relationships (McCauley *et al.,* 2008, Danet *et al.,* 2003). If the mtDNA is transmitted maternally, it tells us that the plant is replicating and changing with its surrounding environment, which increases the fitness of the plant.

By discovering the strawberry plant's ancestry and knowing its patterns of heredity, scientists can better understand the mechanisms of genetics. Also, strawberry breeders can utilize the genetic information to make a strawberry fruit that lasts longer on the produce department shelf or a plant that is disease resistant.

Inheritance in Strawberries

The cultivated strawberry (*Fragaria ×ananassa*) is an important plant in New Hampshire agriculture, and its genetic makeup and complex evolutionary ancestry make it an interesting research subject. My focus was on the heredity of mitochondrial DNA, which had not been studied previously in strawberry. There are two types of cellular DNA: nuclear (which accounts for about 95% of the total DNA in an organism) and organelle (which accounts for the remaining 5% of the total DNA). Mitochondria and chloroplasts are two plant organelles, or parts of the plant cell, and each have their own DNA. Nuclear DNA is inherited biparentally (one copy from each parent), whereas the organelle DNA is usually inherited uni-parentally (from only one parent—usually the female). (Figure 2)



Timmis J. N., Ayliffe M. A., Huang C.Y. & Martin W. Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. Nature Reviews Genetics 5, 123-135. February 2004. doi:10.1038/nrg1271. http://www.nature.com/nrg/journal/v5/n2/images/nrg1271-i1.jpg. April 11, 2010.

Figure 2: Transmission of the chloroplast, mitochondrial and genomic DNA between males and females.

By determining which available parent plants contribute their mtDNA to the offspring of crosses, we can infer the general pattern by which ancestral parents might have transmitted their mtDNA through evolutionary lineages. In animals, the mitochondrial DNA is uniparental and inherited maternally (Stewart *et al.,* 2008, Shoubridge *et al.,* 2007, Ballard *et al.,* 2004). Some plants transmit mtDNA both maternally and paternally through paternal leakage, where some of the paternal DNA shows up in the offspring (Pearl *et al.,* 2009, Wade *et al.,* 2005). However, in most cases plants strictly undergo maternal mtDNA inheritance (Natcheva *et al.,* 2007, Sodmergen *et al.,* 2005). It is known that nuclear DNA is bi-parentally transmitted and the chloroplast DNA is maternally inherited in strawberry. In contrast, the transmission of mitochondrial DNA in strawberry is unknown. My research set out to test the hypothesis that strawberry mtDNA is maternally inherited.

Like in many flowering plants,the cultivated strawberry is polyploid, meaning that it has more than two sets of chromosomes. Specifically, the cultivated strawberry is an octoploid species, meaning that is has eight sets of chromosomes. It evolved from wild, diploid strawberry ancestors (Pontaroli *et al.,* 2009). Polyploidy plays an important role in evolutionary ancestry and contributes to species diversity (Levy *et al.,* 2002, Ayala *et al.,* 2000, Soltis *et al.,* 1999).

In an evolutionary tree, previously separated branches of the tree can merge together when two different species mate, a process known as interspecific hybridization. This often produces new plant species. In animals, interspecific hybridization produces a sterile hybrid (for example, a mule, which results from crossing a horse with a donkey), whereas in plants such hybrids have the potential to be fertile. Fertile hybrids result when an accident in cell division doubles the number of chromosome sets to create a polyploid. Polyploidy often results in increased size and vigor of the plants, perhaps because multiple copies of each nuclear gene are available, enabling one copy to retain the original function while the others can evolve to do new functions (Hartwell *et al.,* 2004).

Previous research indicates that the octoploid strawberry must have arisen from hybridization events involving at least three diploid ancestors. Work in Dr. Davis's lab at UNH has identified two of these ancestors, but the ancestral source of the cultivated strawberry's mitochondrial DNA remains unknown.

Methodology

I started my work on strawberry plants for Dr. Davis by re-potting, labeling, and removing the dead leaves on over 500 strawberry plants in the greenhouse. Later, when I began my mtDNA research project, I learned to isolate DNA from strawberry plants and to perform other molecular techniques. I completed my research in Dr. Davis's lab and I worked closely with Lise Mahoney (a Plant Biology Ph.D. student) under the guidance of Dr. Davis.

I isolated the DNA by crushing strawberry leaves in liquid nitrogen and adding a variety of solutions to "strip" and clean the DNA, thereby leaving only DNA in my sample. It is important to have clean DNA so that subsequent enzymatic procedures such as the polymerase chain reaction (PCR) can work without interference from proteins, RNA, or other substances that would be left in the sample otherwise (Golino et al., 2008). PCR is the technique used to copy specific DNA sequences of interest. In PCR, a high temperature step separates the DNA into single strands, and then the separated strands are used as a template to replicate the DNA, guided by specific PCR primers that search out the mitochondrial or other sequence of interest and prime the synthesis of DNA copies. For my experiment, I used PCR to replicate enough copies of the targeted segment of mtDNA to be visible on an electrophoretic gel. This type of gel, through which an electrical current runs, is used in gel electrophoresis, a technique used to analyze PCR amplification products.

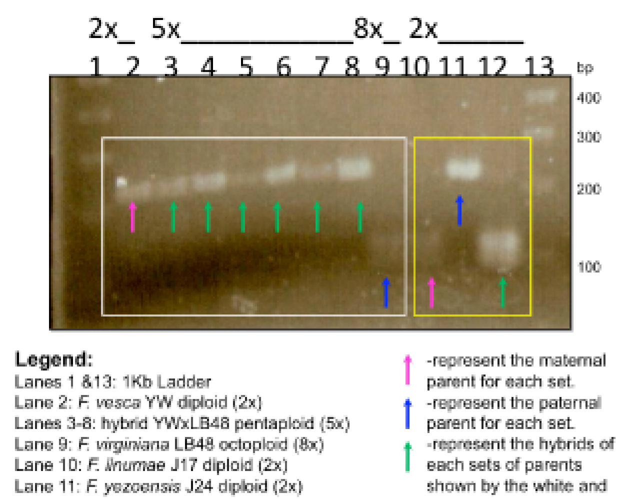
Next, I used a restriction enzyme to cut the mtDNA PCR product at a specific site (CC/GG) that was present in some species' mtDNA but not others. By comparing different species used as parents and the hybrid plants produced by inter-mating them, my objective was to see which parents and hybrids either had, or lacked, CC/GG cuts in similar areas in the DNA sequence. Such DNA sequence differences (polymorphisms) can be used to tell whether certain hybrids received their mtDNA from their maternal or paternal parent.

Because DNA is negatively charged, when run through gel during gel electrophoresis it moves toward the positive electrode. Different segments of DNA move to different places in the gel depending on their number of nucleotides. The larger strands of DNA travel slowly, and smaller strands travel faster. So the uncut mitochondrial DNA PCR product should form a single, slower-migrating band because its length is about 200 base pairs. In contrast, products cut by the restriction enzyme would have two smaller, faster migrating bands,

Conducting research in the greenhouse greatly differed from my experience in the lab, as it was more physical and interactive, whereas the laboratory involved testing at the molecular level. I enjoyed both for different reasons, and with both I was able to visually see an outcome. Because I am in a wheelchair, I faced some physical challenges in the lab, such as an inability to reach certain things and restricted movement around the room. Fortunately my lab colleagues were very helpful with my experiments and made the lab more accessible to me. In addition to the physical challenges, I also faced a lot of trial and error before I mastered PCR and could show presentable results.

Results

Figure 3: Polymorphism in LB48 and J17



By sequencing the PCR products from various strawberry species and their interspecific hybrids, a useful polymorphism was revealed that differentiated the parents of two crosses. The black boxed region in the Figure 3 shows the polymorphism between two different species, diploid *Fragaria iinumae* (J17) and octoploid LB48 (an *F. virginiana* hybrid). A polymorphism strongly suggests that they may have a common maternal ancestor, (Dasmahapatra *et al.,* 2009, Tian X *et al.,* 2006).

Because of this polymorphism, I then tested the hybrids of J17 and LB48. The white box in Figure 3 shows in lane 2 the PCR results for Yellow Wonder (YW), which is the diploid maternal parent of LB48; in lane 9 the results for the octoploid paternal parent LB48; and in lanes 3-8, the results for the six pentaploid hybrids from the cross of YW x LB48. All of the hybrid pentaploid YW x LB48 plants show the same mitochondrial sequence polymorphism as the diploid YW maternal parent. Therefore, this shows maternal transmission of mitochondrial DNA from YW to the pentaploid offspring.

Following the previous knowledge that J17 is likely to be the maternal ancestor, I then studied a cross between two diploid species. The yellow box in Figure 3 shows the PCR results for the diploid maternal parent J17 in lane 10; the diploid paternal parent J24 in lane 11; and the J17xJ24 diploid hybrid in lane 12. The hybrid has the same mtDNA characteristic as J17, which is the maternal parent. This shows maternal transmission of mitochondrial DNA in the diploid hybrid J17xJ24, thereby proving maternal mitochondrial DNA transmission from J17 to the hybrid.

The reciprocal hybrid (J24xJ17) could not be obtained for testing because J24 (*F. yezoensis*)is a self-incompatible species, meaning that it can't successfully pollinate itself (Franklin-Tong *et al.,* 2008), and it also cannot be successfully pollinated by a self-compatible plant such as J17.

Implications

By proving maternal transmission of mitochondrial DNA in two cases of artificial hybridization, YW x LB48 and J17 x J24, my findings will help guide efforts to trace the mitochondrial DNA of the cultivated strawberry back to its diploid origin. Thus, my project made a real scientific contribution by generating new knowledge about strawberry genetics and evolution.

My research as an undergraduate provided me with the unique opportunity to not only conduct an experiment in the lab, but to write grants, present at conferences, and work with graduate students. This experience allowed me to actively put into practice what I was learning in my courses, and by having direct experience working with graduate students, I was able to enhance my undergraduate studies by learning more advanced laboratory techniques.

Since graduating from the University of New Hampshire in May 2009, I now work as an adjunct professor teaching biology at Southern Maine Community College. I thoroughly enjoy teaching and being involved in a college setting. In the future, I plan to attend graduate school for molecular biology and continue to do research with plants.

I want to thank Dr. Tom Davis, Lise Mahoney and the Davis lab, for mentoring me through this project and being committed to the success of my research. I also want to thank the University of New Hampshire for offering an undergraduate research program. This has been an amazing opportunity.

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Author Bio

From Turner, Maine, **Monica Quimby** *graduated from the University of New Hampshire in 2009 with a B.S. in molecular, cellular and developmental biology. She performed research on strawberry genetics from summer 2005 through May 2009, first in the UNH greenhouse and then in Dr. Davis's laboratory. "I was interested in how [strawberry plants] worked beyond the physical characteristics for agricultural purposes," she says. "It was a difficult road because some experiments had to be repeated, but I always learned something new." Monica received funding from the Undergraduate Research Opportunities Program (UROP) in 2009, and went on to present her research at the Northeast Undergraduate Research and Development Symposium at the University of New England and at UNH's Undergraduate Research Conference. Currently, Monica teaches biology at Southern Maine Community College. "Undergraduate research opened more doors and enabled me to explore different avenues," she says. "I now share my undergraduate research experiences when teaching in class." Monica's goal is to attend graduate school and become a biology professor.*

Mentor Bio

Dr. **Thomas M. Davis***, Professor of Plant Biology/Genetics in the Department of Biological Sciences, has taught at the University of New Hampshire since 1984. In both teaching and research, he specializes in genetics, genomics, and evolution; his research focuses primarily on strawberries. As Monica's academic advisor, Dr. Davis learned of her interest in plants and invited her to work for him first in the greenhouse. "She proved to be a productive and responsible assistant, taking care of genetically unique and highly valuable experimental populations of strawberry plants as part of my genomics research program," he says. Dr. Davis has mentored numerous undergraduates, and says that Monica's research was exciting "because she was helping to discover new knowledge that was of immediate significance to my research on genome evolution in strawberry." As her mentor, Dr. Davis was very involved with Monica's writing and revising work as she prepared proposals, presentations, final reports, and now her Inquiry article. "I gained the satisfaction of witnessing the personal and professional growth of a student engaged in real scientific research," says Dr. Davis. "I also gained ongoing inspiration from Monica's indomitable spirit."*