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Exploring the Diversity of Tropical Pumpkin in Costa Rica

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Research Article

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My generation faces the challenge of feeding an exponentially growing population with an increasingly energy-intensive meat diet, while adapting to and mitigating climatic shifts. To engage in this matter, I am graduating with a bachelor of science in sustainable agriculture and food systems (SAFS), a dual-major in international affairs, and a minor in Spanish. Concern about climatic impacts led me to conduct an independent research project in Costa Rica, motivated by my interest in crop diversity and food security. With inspiration from my University of New Hampshire mentor Andrew Ogden, I applied for and received a grant in summer 2016 from the International Research Opportunities Program (IROP) at UNH.

Lizzie Gill

My objective was to assess the genetic and physical diversity of Cucurbita moschata, the species known as butternut squash in the United States and as tropical pumpkin in Costa Rica. (I will refer to this squash species as tropical pumpkin throughout this article.) I set out to explore the variety of tropical pumpkin in local farmers markets (ferias) across Costa Rica and to inventory important characteristics. The relatively tiny country of Costa Rica harbors an amazing 5 percent of the world's biodiversity and is located directly in the center of origin for squash, with ties to ancient hardy varieties. I spent the core of my nine-week research visit at the Fabio Baudrit Agricultural Station (FB), a research station and campus of the University of Costa Rica located in the Central Valley, where 70 percent of Costa Ricans live. This rich valley is known for its temperate, hospitable living conditions and agricultural activity. The Fabio Baudrit was the base location for my collection trips around the country and a place to perform inventory assessments. I lived with a wonderful Costa Rican family, whose home cozied up to the fragrant panadería (local bread store). It was a ten-minute run from acres of sugar cane and coffee plantations and a fifteen-minute walk from the famous steel Nuestra Señora de las Mercedes Catholic church in the vibrant city of Grecia. Here, I was able to refine my Spanish language skills and experience the culture through a local perspective. I found a country rich in plant biodiversity worthy of being studied and preserved for the future of our planet.

Agriculture, Tropical Pumpkin, and Costa Rica

Current agricultural practices require high yields and hardy products for customers demanding high-quality, low-priced goods. This standard has been achieved through years of crop domestication, trade, selection, and research starting around 10,000 years ago from the eight genetically diverse centers of origin named by Russian botanist and geneticist Nikolai Vavilov (Vavilov, 1935). These eight centers (South Mexican and Central American, South American, Mediterranean, Middle East, Ethiopia, Central Asiatic, Indian, and Chinese) are believed to be the original locations for the domestication of current agricultural crops, and contain a rich diversity of wild relatives with a variety of traits. Over time though, the human selection of crops from these areas to fit into efficient, large-scale monocrop systems has decreased commercial crop diversity, making many agroecosystems vulnerable. This vulnerability poses a huge threat to food security. In contrast, increased biodiversity has been shown to vastly increase agroecosytem health, resistance, and adaptability (Altieri, 1999). As Liberty Hyde Bailey, a key figure in horticultural and botanical history, notes, a crop must maintain variation in order to have its full range of potential in differing environments (Kingsbury, 2009). Breeders can use different traits to improve crop characteristics like nutrition, yield, and disease resistance (Hawkes, 1997).

The diversification of the genera *Cucurbita*, commonly known as squash, pumpkins, and gourds, is an example of a commercial crop that can increase agroecosystem health through maintenance of its trait diversity. *Cucurbita* are one of the most economically significant genera, ranking in the top 10 most important vegetable crops worldwide (Ferriol and Pico, 2008). This includes the United States, which imports the most squash in the world, averaging around $339.7 million worth of product in 2016 (USDA Economic Research Service, 2017). *Cucurbita* also account for around 50 percent of total fresh vegetable production in the developing world (Esteras et al., 2008). Local, small-scale farmers use the fruit and flower as a food source and the mature seeds for the following year's crop. Recently, developing countries have also increased large-scale production and export of new cultivars (Andres, 2004; Robinson and Gneva, 1995). Within the *Cucurbita* genera, tropical pumpkin, or butternut squash *(C. moschata),* is one of the more economically significant varieties (Rodriguez-Amaya, 1999). Tropical pumpkin are uniquely high in carotenoids and vitamin A, which have anti-inflammatory properties, aid disease prevention, and can improve immune function, vision, reproduction, and cellular communication (Rodriguez-Amaya, 1999). These economic and nutritional benefits raise the importance of continued research on *Cucurbita* crops.

Tropical pumpkin sample

Like many conventional crops, the true region of origin for *Cucurbita* is still uncertain. Central America is one of the potential centers of origin for *Cucurbita* (Sanjur et al, 2002). This means that high levels of variation in traits such as color, shape, size, sweetness, and texture can be found among the genera in this location. Of particular interest to plant breeders is the increased resistance to biotic and abiotic stresses often found in wild varieties. Originally, Native Americans domesticated tropical pumpkin with high genetic diversity in the three sister system (maize, bean, and squash). In the fifteenth and sixteenth centuries, when the Spanish and Portuguese colonized parts of Central America, only select varieties were brought back to Europe (OECD, 2012). These cultivars were manipulated and bred for colder climates with long-day or short-night flowering, which resulted in today's classic, strait-necked butternut varieties sold in the United States (OECD, 2012). This variety of seasonal butternut squash is physically very different from the warm-climate, short-period tropical pumpkins produced in Central and South America, yet their similar genetics allow for successful crossing. Therefore, the two varieties are considered the same species, *C. moschata.*

Unfortunately, with recent climatic shifts and large-scale industrial agriculture, much of the native biodiversity and germplasm (the genetic material available for a plant breeder to work with) is disappearing at a fast rate (INBio Costa Rica, 2014). The diverse traits found in Costa Rica must be catalogued soon to preserve biodiversity. Breeding programs can then use varieties that possess these traits to produce plants with superior disease resistance, climate hardiness, increased nutritional value, and many other traits that increase economic value (Hawkes, 1997).

At the University of New Hampshire, Professor Brent Loy has developed many new beneficial and profitable *Cucurbita* varieties. One of his projects focused on breeding for increased carotenoid content in *C. moschata* (Noseworthy and Loy, 2012). His research shows the importance of exploring and having access to diverse crop germplasm. Ideally, my research, which aimed to archive this important tropical squash germplasm, would help conserve biodiversity and lead to more adaptable squash varieties—ultimately improving the sustainability and efficiency of food systems in both the United States and Costa Rica.

The Two-Part Process

Creating an inventory involved two parts. First I compiled a diverse set of tropical pumpkin samples from *ferias* in different regions of Costa Rica. I spent two to three days each weekend traveling in an old University of Costa Rica truck with Geovani, a worker from Fabio Baudrit. We motored around the country from dry and hot to tropical and mountainous climates. The bustling *ferias* varied from small, street-side displays to football-field-sized venues exploding with local farmers. Farmers threw samples of fresh produce our way to make dents in their infinite piles of pineapple, tomatoes, and coconuts. The towering mountains of colorful produce were often accompanied by the sound of live marimbas and the smell of the freshly fried *chicharrón*, or pork belly. At each venue, I asked the vegetable vendors for *ayote*

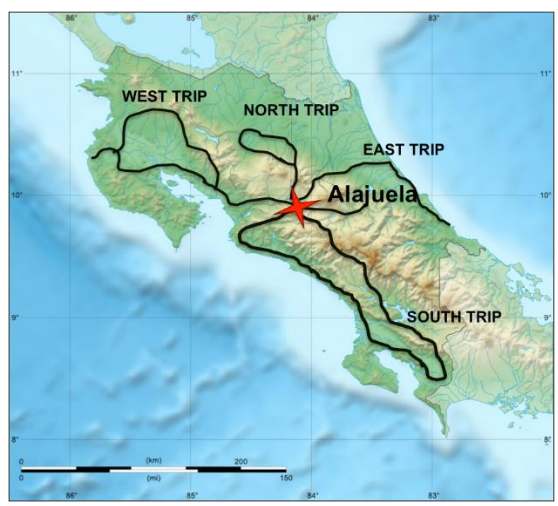

Figure 1: Outline of the long collection trips to differing regions of Costa Rica. (*Map courtesy of www.nationsonline.org)*

sazón. This is the Costa Rican term for mature tropical pumpkin, distinct from the *ayote tierno*, which are immature *ayotes* used as summer squash in their culinary application. I then purchased as many unique varieties of tropical pumpkin as I could find. I made five longer trips around Costa Rica and four shorter trips within the Central Valley. The five long trips included trips west (Puntarenas, Nicoya, Liberia, Santa Cruz, and Cañas), south (Uvita, San Isidro, Ciudad Neily, San Vito, and Buenos Aires), north (San Ramon, La Fortuna, and Quesada) and east (Cartago, Turrialba, Siquirres, Limon, and Puerto Viejo) (Figure 1). The local trips in the Central Valley included San José, Alajuela, Grecia, Sarchi, Naranjo, and Heredia.

For the second part of the inventory process, I assessed collected samples during the workweek. In the Laboratory of Cellular and Molecular Biology at the Fabio Baudrit Agricultural Station, I measured and recorded thirty-five physical characteristics of each sample of tropical pumpkin. These included shape, circumference, weight, color, and strength of pulp and skin, along with dry weight and Brix measurements. Dry weight determined the starch content, which is a good indicator of the eating quality and texture of the fruit. Brix measurements determined the level of soluble sugars, or sweetness. For each sample of tropical pumpkin, I also took five photos (to include full fruit, cross-section, seed, plant, and plant leaf); dried and vacuum-sealed 50 seeds; and performed a cooked taste test. After I recorded all the phenotypic information, we grew three to four seeds from each sample for one and one-half weeks to acquire leaves for DNA analysis. With the help of my mentor, Walter Barrantes, a professor and investigator in molecular biology at the University of Costa Rica, I learned the process called phenol-chloroform DNA extraction. This is a method that isolates the tropical pumpkin DNA. Unfortunately, due to the short term of my project, I only was able to extract DNA from my samples and did not have time to do further analysis of the extracted DNA. In the future, Dr. Barrantes will screen for mutations in the genetic material using high-resolution melting. This will allow him to correlate marked differences in the DNA to the phenotypic variation that I observed and cataloged.

Obstacles

My research came up short of my objectives due to a few obstacles. First, I was able to collect only 43 of the planned 100 tropical pumpkin samples due to seasonal hindrances and the unexpected entrance of new hybrid varieties. *Granjeros,* or Costa Rican farmers, sow most of their tropical pumpkin seeds during June, July, and August, making peak harvest during the months of December, January, and February. I performed my research during the major planting season (not harvesting), which reduced availability of squash in the markets at that time. With that said, the collections were still valuable and the number of samples far outnumbered the number of varieties I would find in US markets. The bigger obstacle I faced was the surprising presence of two new, imported, hybrid seed varieties of tropical pumpkin (Arjuna F1 and Leela F1) that have only recently started to flood the Costa Rican markets. These seeds are purchased through East-West Seed International, a company based in Thailand that conducts intensive breeding programs to develop improved hybrid vegetable crops. Both the Arjuna F1 and Leela F1 hybrids contain a high flesh-to-size ratio, a good taste, a consistent weight and shape, and a long shelf life. Costa Rican farmers have quickly transitioned into selling primarily these varieties. This made it much harder to find the variety of tropical pumpkin I had anticipated for this project but also shows an interesting shift occurring from cultural agricultural methods to more conventional methods in Latin America.

What to Take Away

The forty-three samples I collected indicate high diversity among tropical pumpkin in Costa Rica. The fluctuation of four economically significant physical characteristics (Brix, percent dry matter, color, and weight) in six of the seven Costa Rican provinces reveals a great variety of tropical pumpkin in the country (Figure 2). The diversity of these characteristics deserves to be cataloged and used to save biodiversity for future crop breeding and improvement. Figure 3 shows percent dry matter of tropical pumpkin in six Costa Rican provinces. San Jose samples have significantly higher dry matter content. This knowledge gives breeders a fast way to locate and select crops with this desired characteristic. With results like these, the plant breeding process could be accelerated in Latin American countries. Indeed, as expressed earlier, there is a significant shift from local farmers saving seeds to purchasing international, hybridized seeds for planting. A uniformly shaped, sized, and fleshed variety is easier for farmers to grow, transport, and store compared to the local varieties with large deviations in these characteristics. Evidence of use of international hybrid seeds emphasizes the potential market for locally bred seed varieties as well as a greater need to collect and preserve species biodiversity.

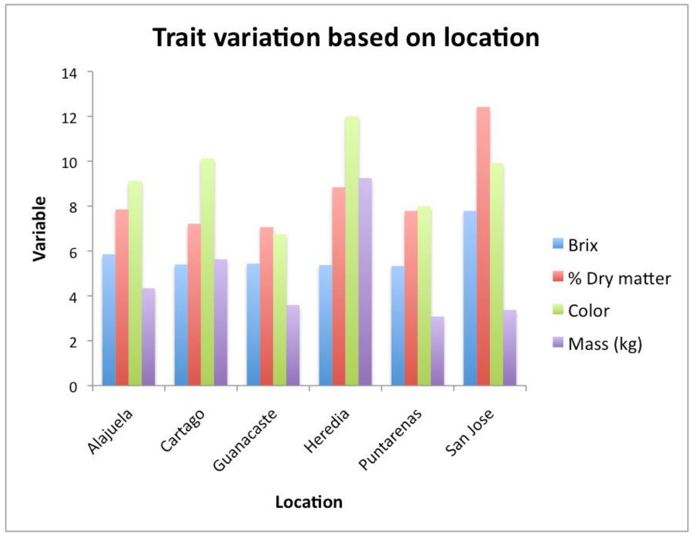

Figure 2. Diversity of four important physical characteristics based on location in Costa Rica. Brix represents sweetness; dry matter is a good measure of starch content or texture; color can be correlated with nutritional content, and weight serves as a parameter to assess yield. Data Analyzed using the statistical software, JMP version 12.

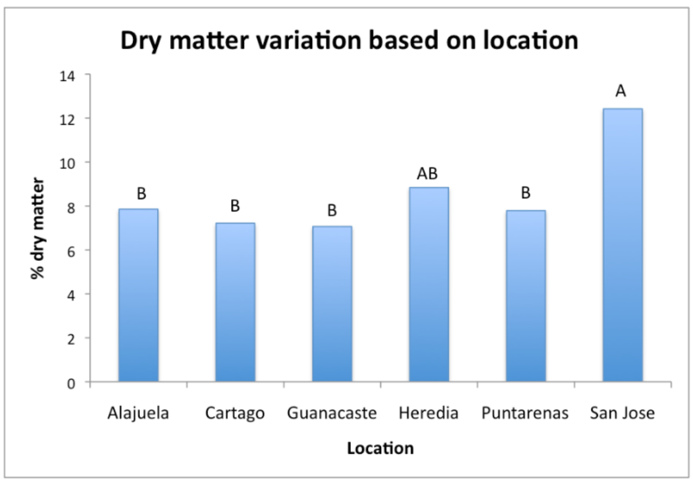

Figure 3: Diversity of dry matter measurements based on location. Single factor ANOVA analysis determined that location did affect squash dry matter content. Squash collected in San Jose region show higher dry matter content than all other regions except Heredia. Data analyzed using the statistical software, JMP version 12. Bars that share a letter in common are not significantly different at $P<0.05$ according to Tukey's HSD test.

The main take away is that there is still tropical pumpkin diversity in Costa Rica and that the samples I collected and the traits they hold are now being preserved as seed packets at the Fabio Baudrit Agricultural station. Plant breeders can use these seeds to produce improved, more adaptable varieties. It also means that we can capture this biodiversity before it is possibly destroyed through environmental change. Better-adapted tropical pumpkin varieties can produce more sustainable, resistant, and efficient agricultural food systems in both the United States and Costa Rica. Ultimately, this can positively benefit the farmer and the consumer.

Appreciation

I returned from Costa Rica with solidified Spanish language skills, improved research methods, new international ties, a fresh perspective, and new confidence. I acquired a great sense of the country through all of my collection trips. The combination of living with a non-English-speaking host family and performing research in Spanish made a trip that could have been very sheltered into a fully immersed cultural experience. I still miss my 9:30 a.m. café with the secretaries at the Fabio Baudrit station and I know rice and beans will never taste as good as they did during that nine weeks. Thanks to the diversity of tropical pumpkin in Costa Rica, not only did I learn to prepare eleven new squash dishes and obtain a new respect for the beauty of crop diversity, but I also made lifelong connections. By the end of my travels, my colleagues at the station referred to me as *gringa de ayote*—the American squash girl. Even though I will always be a *gringa,* I was lucky to have a sneak peak into the Tico (Costa Rican) lifestyle.

This experience heightened my feeling that there is great potential and opportunity for change in the world. I have a stronger belief now that we can all contribute to the critical work my generation faces globally. I have a passion to play a role in improving sustainability (economic, social, and environmental) and in making shifts in global agricultural practices with the least amount of harm to the environment. I know the processes that influence farm-to-fork are complex, but I hope that by focusing on local problems in the United States and in Latin and South America we can make meaningful change. As for my next steps, I am shipping off to Paraguay after graduation to serve as a Crop Extension Peace Corps Volunteer with the goal of assuring food availability and income generation for small-scale rural farmers through the implementation of sustainable food practices.

This research would not have been possible without the generous donations of Mr. Dana Hamel, Mr. & Mrs. Noonan, and Mr. Carlton W. Allen through the UNH Hamel Center International Research Opportunities Program (IROP). Thank you for all the hard work of the Hamel Center staff, especially that of Georgeann Murphy and Peter Akerman. Finally a big shout-out to my UNH mentor, Andrew Ogden, and my mentor abroad, Walter Barrantes, for leading me through my first independent undergraduate experience.

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Author and Mentor Bios

Lizzie Gill will graduate from the University of New Hampshire in May 2017 with a dual major in sustainable agriculture and food systems and international affairs and a minor in Spanish. She is a member of both the University Honors Program and the Honors in Major Program. Lizzie grew up in Montana, where she came to love the outdoors and her unique natural surroundings. A strong believer in systems thinking, Lizzie is trying to use her agricultural studies to construct a bridge between biological and sociological sciences to address global food insecurity and environmental degradation. This past summer, she traveled to Costa Rica through the support of a Hamel Center International Research Opportunities Program (IROP) grant to perform independent research on the diversity of squash vegetables. She enjoyed the challenge of working with another culture in a different language and dealing with different cultural norms in the scientific and nonscientific world abroad. After graduation, Lizzie will continue pursuing her interests by traveling to Paraguay, where she will serve as a Crop Extension Peace Corps volunteer until December 2019.

Andrew Ogden has taught at UNH for five years and currently is a lecturer in sustainable agriculture and food systems in the Department of Biological Sciences. Lizzie is the third student he has mentored for research in Costa Rica through Hamel Center research grants, but she is his first mentee to write for *Inquiry*. Ogden has a strong connection to Costa Rica. He has owned and operated an educational center and farm there since 2002; he teaches an agriculture and development J-term course based entirely in Costa Rica; and he has a strong network of horticulture colleagues in the country. Like Lizzie's foreign mentor, Dr. Walter Barrantes, his research focuses on breeding and physiology of cucurbit crops. Ogden says that through Lizzie's research, which she "embraced…from start to finish," he gained a much better understanding of the types of squash found in Costa Rica. Ogden is working on his PhD under the directions of Dr. Brent Loy. He encourages any student interested in studying in Costa Rica to contact him at UNH.

Walter Barrantes Santamaria agreed to mentor Lizzie Gill at the request of her UNH mentor, Andrew Ogden, and he hopes to work with other students like Lizzie in the future. Dr. Barrantes greatly appreciated Lizzie's contribution to the work at the Agricultural Experiment Station, Fabio Baudrit Moreno University of Costa Rica, where he is an agronomist and molecular biology lab coordinator specializing in molecular breeding. He provided logistics support to Lizzie (lodging, lab access, etc.) and trained her in molecular lab techniques related to her research on tropical pumpkins. Dr. Barrantes commends Lizzie's professional performance on the project and found being a mentor a gratifying experience.