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Unraveling the Tapestry of Indigenous Maize in North America: A Case Study of Pawnee Ancestral Maize

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UNRAVELING THE TAPESTRY OF INDIGENOUS MAIZE IN NORTH AMERICA: A CASE STUDY OF PAWNEE ANCESTRAL MAIZE

by

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UNRAVELING THE TAPESTRY OF INDIGENOUS MAIZE IN NORTH AMERICA: A CASE STUDY OF PAWNEE ANCESTRAL MAIZE

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Studies on Indigenous ancestral landrace maize in North America has significant historical and scientific importance. Indigenous peoples, such as the Pawnee people, have been cultivating maize for thousands of years, resulting in diverse varieties adapted to their local environments. This study aims to deepen the knowledge of Indigenous maize by examining specific varieties from the Pawnee, including a comparative analysis of the genetic makeup through DNA sequencing. This study used Genotyping by Target Sequencing (GBTS) method to examine the genetic variation and characteristics among the multiple varieties the Pawnee people once grew historically, providing valuable information about the evolutionary history and genetic diversity within a species. By sequencing various DNA samples of maize varieties cultivated from the Pawnee people after years of selective breeding and observation in their homeland of Nebraska, we can gain insight into the genetic characteristics and understand the factors that have influenced the diversity in maize even grown within the same region by the same people. The comparison between Indigenous maize DNA and contemporary maize DNA will enable us to identify genetic variations specific to these Indigenous maize varieties. Studying the genetic diversity of Indigenous maize is consequent for conservation efforts, allowing us to identify and protect specific varieties. This research contributes to our understanding of evolutionary history and genetic relationships within the maize species.

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ACKNOWLEDGMENTS

Firstly, this project would never have happened without my mentors, Deb Echo-Hawk and Ronnie O'Brian, encouraging me to continue my work and studies at the University of Nebraska. I am immensely grateful for their trust and faith in my abilities, and their ever-present joy and excitement for Pawnee corn.

These precious ancestral seeds would not have returned to the Pawnee people in such abundance without the hard work of our trusted Nebraska gardeners. I would like to especially thank the Nebraska growers from the Pawnee Seed Preservation Society. I have gotten to know those that tend these crops every year since I began this work and would like to thank them all again for the work they do: Bill Bolte, Nate Belcher, Jerry Carlson, Marlan and Sherrie Dux-Ideus, Del Ficke, Pat and Steve Hogeland, Gene Hunt, Crystal Hunt, Brad Kindler, Lyn Krabiel, Ronnie O'Brien, Tina Smith, and Roger Woolsey. If I have forgotten any growers, please forgive my tired mind.

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So many people have stepped in to help me during the completion of this project. Help with DNA statistical analysis was provided by graduate student Musa Ulutas and advisor Jinliang Yang. I am also very grateful for the assistance of my current committee members Jinliang Yang, James Schnable, and Thomas Hoegemeyer, and L.J. McElravy. Thank you to the UNL Department of Agronomy and Horticulture and the professors who offered assistance and understanding to me

throughout my research. It could not have been accomplished without their support.

Without any less appreciation and the most reverence, I want to thank and acknowledge my ancestors. This research and my existence would not be possible without my Pawnee ancestors that survived to continue our Pawnee traditions.

INTRODUCTION

In the United States, there are currently close to 600 different Native American tribes recognized by the federal government; these are all different tribes, with different languages of their own and cultures and practices from each tribe. But currently there are only a few million Native Americans today, which is just over 2% of the US population, according to the 2020 U.S. Census (U.S. Census). Many of these Native American tribes now are small in numbers and have lost a lot of information about their own languages, cultures, and practices; not only because of this huge loss in numbers but the few survivors were also taught to renounce their cultures and practices. It has only been a few generations of Native Americans living now that are trying to relearn their culture and restart these practices. It is a battle even now to get people to appreciate these attempts to bring back their own culture. The research conducted in this study aims to understand the ancestral crop of a tribal community, endeavoring to contribute to seed and culture preservation efforts.

History of the Pawnee People

"Pawnee are the people of the buffalo and the corn," according to the Pawnee Nation. In the 18th century more than 60,000 members of the Pawnee tribe had permanent earth lodge settlements in the Nebraska territory area (Pawnee Nation, 2023). After the Pawnee had ceded their territory to the U.S. government in the 1800s and were removed from Nebraska to a territory in Oklahoma in 1875, the number of Pawnee members was down to just over 600 in the 1910 census count (Pawnee Nation, 2023). Before leaving Nebraska and upon entering Oklahoma, Pawnee survivors were taught to renounce their cultures and practices. Like many other tribes, the Pawnee stopped their agricultural

practices on the less fertile land they were forced on to, and almost lost their seeds and knowledge. Today the Pawnee Nation of Oklahoma has around 3,500 enrolled members and can be found in all areas of the United States. There has been a renewal in interest in Pawnee agriculture over the last few decades with the growth of the Pawnee Seed Preservation Society (PSPS) in Pawnee, Oklahoma.

Review of Literature

Pawnee Agriculture History

To understand the history of Pawnee Agriculture and the importance of maize to the Pawnee people, Gene Weltfish's *The Lost Universe* is referenced, "To the Pawnee, the ear of corn was his daily food and also his most profound reverence and worship" (Weltfish, 1965). Maize was not only an important part of the Pawnee diet but revered as a sort of holy entity with several ceremonies focused on maize including the Mother Corn ceremony, the Young Corn Plant ceremony, the Great Cleansing ceremony, and several Harvest ceremonies (Murie, 2012).

As Weltfish elaborates, "Without this ceremonial cycle, the coordination of the people in their orderly round of life could not go on. While it was primarily a religious frame, it was also a basic practical value since it was based on the seasonal progression" (Weltfish, 1965). The timing of the crop's life cycle determined when encampments were moved, when hunting parties could leave, and daily work for many Pawnee women, as well as the proceedings of almost all Pawnee ceremonies. Pawnee ceremonies contained bundles that "represented cosmic powers there were two ears of corn or an ancient breed, cultivated exclusively for its role as a holy object" (Weltfish, 1965).

The Pawnee's other major crops were squash and beans, of which they also had several varieties consumed as part of their main source of nutrition. Although those crops domesticated into the new world before maize, the Pawnee placed no religious connotations to these crops as they had with maize. The Pawnee also were not observed to have as much reverence for their cultural practices outside of agriculture as other

peoples did, "…there were neither religious taboos nor ceremonial procedures connected with the actual practice of crafts" (Weltfish, 1965). Weltfish goes on to list a number of tribes in North America and also other peoples around the world that have strict taboos and ceremonies regarding practices such as bead work, pottery, basket making, building making. The Pawnee did not hold crafts to such strict procedures although "…their work was as fine and precisely done but there were no religious connotations of any kind" (Weltfish, 1965). This is to note just how important their maize was to the Pawnee. Maize grown by the Pawnee tribe was not only revered by the Pawnee people and those specially studying Pawnee maize, but Pawnee maize is noted by other tribes and other old studies on other Native American tribes. Atkinson and others studying Native American tribes of Montana in 1915 claims, "The Pawnee and Wichita are speculated to have brought their maize crops to different regions. They are believed to have been the first to raise maize in the upper Missouri" (Atkinson, 1915). It remains to be discovered as to which peoples grew maize in the upper Missouri region first; but with more research and technologies becoming available for study, this debate may be concluded soon.

Pawnee Seed Preservation Society (PSPS)

The PSPS continues to grow Pawnee seed to this day, helping the Pawnee people to reclaim their agricultural heritage. The Pawnee Seed Preservation Project (Society) began in 1998 when tribal member Deb Echo-Hawk began her role as Keeper of the Seeds for the Pawnee people by gaining approval from the Pawnee Culture Committee and Pawnee Nasharo Council of Chiefs; then gathering and growing three varieties of Pawnee corn preserved by Pawnee families (Echo-Hawk, 2020). After Rhonda O'Brien joined the PSPS in 2003, beginning the growth of Pawnee seeds in Nebraska again, the Pawnee seed

bank began to grow and varieties that “were lost for over 146 years, for reasons you can imagine (tribal relocation, boarding schools, changing of style and farming equipment)” (Echo-Hawk, 2020).

The PSPS began using an old research study by Will and Hyde from 1916 examining maize grown by Native Americans along the upper Missouri River (Will and Hyde, 1917). This study included measurements, photographs, and observations of how these varieties were grown by their corresponding tribes. With this data and the help of experts interested in the revival of Native crops, the PSPS began focusing on breeding their maize varieties to match that of older generations as recorded by Will and Hyde (Echo-Hawk, 2020). At the time of Will and Hyde’s study, they identified 9 “pure” strains of Pawnee corn. This corroborates with the Lost Universe, as Mark Evarts identifies 10 corn varieties the Pawnee were growing, 9 being of Pawnee varieties and 1 Osage variety (Weltfish, 1965). As of 2022, the PSPS claim to have close to 20 Pawnee maize varieties that were once grown by the Pawnee in Nebraska.

The Pawnee people did not grow their revered varieties for around 100years. After losing many of their seeds and agriculture practices from relocations to Oklahoma and further losing their culture practices in forced enrollment to boarding schools in Nebraska and Oklahoma; there are some beneficial considerations. As Galinat points out in his late study of maize and culture evolution in America, “Indigenous people of North America selectively bred wild maize into the agriculture-dependent version we know today. Maize fruiting, seeding and pollinating processes were considerably changed by their cultivation of the crop” (Galinat, 1965). The Pawnee people almost lost their maize varieties completely, by not growing them during this period of industrialization in agriculture

practices. Their maize varieties did not change much in characteristics when they were finally grown and bred again generations later. That is why the PSPS have relied heavily on the scientific observation and research that Will and Hyde conducted over 100 years ago, the last that some of these Pawnee maize varieties were seen.

Objectives

The primary goal of this project is to genetically classify and further study Pawnee landrace maize. This will help with the development of a comprehensive understanding of North American landrace maize, begin a comparative baseline of maize information for the Pawnee Nation of Oklahoma. This data will be documented for the Pawnee Seed Preservation Society to continue future genetic research into these landrace maize varieties.

Landrace maize varieties have a wide range of diversity in its morphological traits. This study will help to better classify Pawnee varieties for future identification of maize varieties; thus, expanding the understanding of Pawnee culture, landrace relationships, and maize diversity.

Previous studies have identified up to 15 different Pawnee maize varieties as they were grown historically in Nebraska (Will and Hyde, 1917). The results of this study will be compared to those of previous studies to confirm and clarify differences among these Pawnee varieties.

Sweetcorn varieties were included in this study and served as controls. Only their DNA material and phenotypic characters were examined. Understanding the relationship

between these landrace maize varieties and current maize accessions can provide insight into relationships of maize and the peoples growing it.

Phenotypic Data Collection

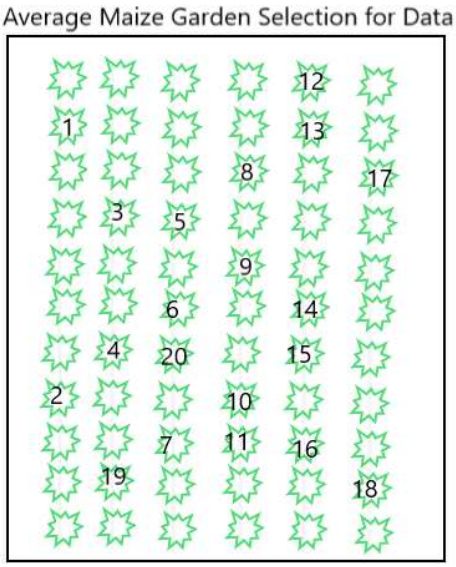
Field experimental design for phenotypic data collection

Beginning in 2017, the PSPS began collecting data on their landrace ancestral maize grown in Nebraska. The data sheets used were assembled with aid from maize breeding expert Tom Hoegemeyer, professor at the University of Nebraska Lincoln, in order to protect these maize varieties in the future. The observed and measured characters match the requirements for U.S. patent and plant variety protection systems. The PSPS has continued to collect that same data every growing season from their ancestral landrace maize to this day. That data was used to help analyze and determine which Pawnee maize varieties to be used for this study. The data used in this study I personally collected each year to minimize variation in technique or judgment.

A typical maize garden grown for PSPS in Nebraska that was used for the data collected in this study was approximately 12ft x 20ft and held approximately 250seeds of one Pawnee variety in each garden (Figure 1). Data was collected from 20 randomly selected plants in each garden for the plant characteristics, as shown in Figure 2, and 20 randomly selected ears and kernels for the ear and kernel section from each garden.

Figure 1: A typical garden for PSPS where data was collected. An average size maize garden for PSPS had ~250seeds planted.

Figure 2: Example of random selection for typical maize garden grown by PSPS gardeners for data collection.



The first set of data was collected when the maize was in pollenating stage. I estimated when each garden was 50%silked and roughly 50%pollen shed on the tassels. These times did not always align, and that time difference was noted when collecting data. All data collected in this stage was done on site for each garden.

The next set of data was collected after harvesting all ears and nubbins from the corn stalks and after ears were dried. This data was collected in the office of PSPS headquarters in Oklahoma, again by me personally.

Traits Explained

As mentioned before, the traits that were measured were determined with assistance from maize expert Tom Hoegemeyer with the intent to patent or protect these maize varieties in the future. This section will briefly explain the traits that were measured. So as to not confuse how the main stalk with the tiller and ear leaf were identified on a typical maize plant, refer to Figure 3. A total of 38 traits were categorized into four groups, including 4 phenology traits, 21 vegetative traits, 8 ear traits, and 4 kernel traits (see Table 1 for details).

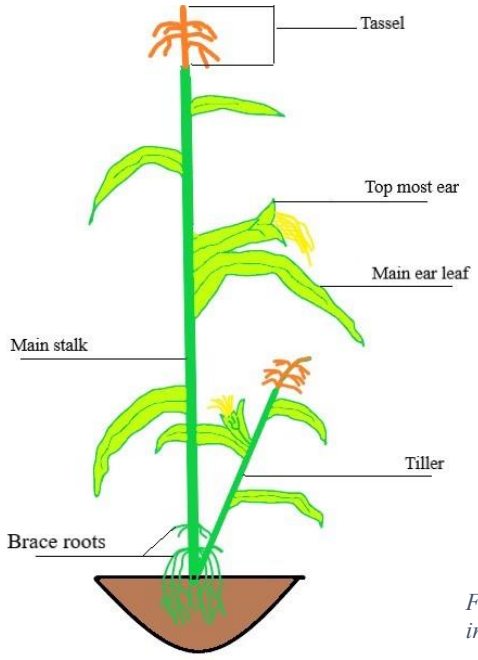


Figure 3: Main maize measurements for made in this study.

The first set of data included measurements of the maize plant, from the main stalk. Plant height was measured from the ground at the base of the main stalk to the top of the main

tassel. Ear height was measured from the ground at the base of the main stalk to where the topmost ear connected to the stalk. Internode length was measured from the topmost leaf before the tassel began to the second topmost leaf. The number of tillers was measured by counting the extruding branches that came off the main stalk at or below ground level. The number of ears was measured by counting the number of ears already forming on the main stalk of the plant and did not include nubbins or ears from the tillers. Purpling brace root was measured by observing the color of the brace roots and scored on a 1-4 scale, 1 being the absence of purple color and 4 being a dark purple color present on the brace root.

The leaf characteristics measured were measured from the main leaf coming off the topmost ear on the main stalk of the maize. Width of ear leaf was measured by finding the widest part of the leaf and measuring across to the nearest ¼ inch. Length of ear leaf was measured from the tip of the leaf to the start of the leaf, or where it attached to the ear, to the nearest ½ inch. The number of leaves was measured by counting the number of leaves on the main stalk above the topmost ear on the main stalk. Leaf pubescence was measured by observing the tiny hairs on the leaf and scored on a scale of 1-9; 1 having no hairs and being completely smooth to 9 having many hairs and being peach-like. Leaf shade was measured by observing the color of the leaf on a scale of 1-4; 1 being a light green color to 4 being a very dark green color.

Tassel characteristics were measured from the tassels on the main stalk of the maize plant. The number of branches was measured by counting the branches that came off the main tassel branch. Length of tassel was measured from the bottom of the tassel, where the topmost leaf starts the tassels, to the top of the central tassel. Peduncle length was

measured from the topmost leaf to the bottom most branch of the main tassel. Tassel branch angle was measured by observing the angle of the central tassel on a scale of 1-3; 1 being an upright central tassel, 2 being a horizontal central tassel, and 3 being a drooping central tassel below the horizontal line. Central spike length was measured from the top of the central tassel branch to the bottom of the central tassel branch. Pollen Shed was measured by observing the amount of pollen coming from the tassels on a scale of 0-9, 0 being no pollen at all to 9 being heavy pollen shed on all branches of the tassel. Anther color was measured by overserving the color of the majority of the anthers on the tassels and scored 1-9; 1 being green or yellow, 3 being pink, 5 being red, 7 being dark red, and 9 being purple. Glume color was measured by overserving the color of the majority of glumes on the tassels and scored 1-9; 1 being green or yellow, 3 being pink, 5 being red, 7 being dark red, and 9 being purple.

Unhusked ear characteristics were measured on the plant and at the same time all other plant characteristic data was collected. Ear data was taken from the topmost ear on the main stalk of each maize plant. Silk color was measured by observing the overall color of the silks on an ear and scored 1-9; 1 being green or yellow, 3 being pink, 5 being red, 7 being dark red, and 9 being purple. Husk color was measured by observing the overall color on the ear and scored 1-9; 1 being green or yellow, 3 being pink, 5 being red, 7 being dark red, and 9 being purple.

Husked ear characteristics were measured after harvested and dried, in PSPS offices. Ear length was measured from the top of the ear to the bottom of the ear to the nearest ½ inch. Ear diameter was measured by first finding the circumference of the ear by wrapping a string around the widest part of the ear, marking where the string meets, and

measuring that length to the nearest ¼ inch. After data was collected, I then divided the circumference collected by pi and rounded it to the nearest hundredth. Ear weight was measured using a digital scale to the nearest hundredth gram. The number of Kernel rows was measured by counting the rows around the ear. The number of Kernels per row was measured by counting the number of kernels up one row from the bottom of the row to the top of the row on an ear. Kernel rows were measured by observing the rows around the ear and determining if the rows were overall distinct from each other and scored a 2, or indistinct from each row and scored a 1. Row pattern was measured by observing the overall pattern on the ear and scoring the ear 1 for a straight patter, 2 for a slightly curved pattern, and 3 for a spiral pattern.

The kernel characteristics were measured after harvested, dried and either taken from 20 random ears from each garden or taken after all kernels were removed from the ear for each garden and randomly picked. Kernel length was measured from the bottom of the kernel to the top of the kernel to the nearest millimeter. Kernel width was measured at the widest part of the kernel from one side to the other to the nearest millimeter. Kernel thickness was measured by turning the kernel on its side and measuring from one side to the other to the nearest millimeter. Color pattern was determined by observing the overall color of the kernel, determining if the color is all the same and scoring it a 1, or determining if there are segregating colors on the kernel and scoring it a 2.

Table 1: Maize traits recorded by PSPS and their descriptions.

Variable	Variable code	Variable description
Phenology		

Planting Date	Pdate	The date when seeds were put into the outside ground
Silking Stage	Sstage	The date when silk appeared on 50% of plants in a garden
Pollinating Stage	Pstage	The date when pollen appeared on 50% of plants in a garden
Harvest Stage	Hstage	The date when mature ears were taken from the plant
Post Harvest Stage	PHstage	After ears were dried and kernels separated from the ear
Vegetative Characteristics		
Plant height (in)	PH	Measure from the ground at the base of the main stalk to the top of the main tassel
Ear height (in)	EH	Measure from the ground at the base of the main stalk to where the bottom most ear connected to the stalk
Internode length (in)	IL	Measured from the top most leaf before the tassel began to the second top most leaf
Number of Tillers	NoT	Count the extruding branches that came off the main stalk at or below ground level
Number of Ears	NoE	Count the number of ears already forming on the main stalk of the plant
Purpling Brace Root (scale 1-4)	PBR	Observe the color of the brace roots and scored on a 1-4 scale, 1 being the absence of purple color and 4 being a dark purple color present on the brace root
Leaf		
Leaf width (in)	ELW	Measure at the mid-point of the primary ear leaf, to the nearest 1/4inch
Leaf length (in)	ELL	Measure from the tip of the leaf to the start of the leaf, or where it attached to the ear, to the nearest ½ inch
Number of leaves	NoL	Measure by counting the number of leaves on the main stalk above the top most ear on the main stalk
Leaf pubescence (Scale 1-9)	ELP	Observe the tiny hairs on the leaf and scored on a scale of 1-9; 1 having no hairs and being completely smooth to 9 having many hairs and being peach-like
Leaf Shade (Scale 1-9)	ELS	Observe the color of the leaf on a scale of 1-4; 1 being a light green color to 4 being a very dark green color
Tassel		
Number of branches	NoB	Count the branches that came off the main tassel branch
Tassel length (in)	TL	Measured from the bottom of the tassel, where the top most leaf starts the tassels, to the top of the central tassel
Peduncle length (in)	P	Length from the collar of the top leaf to the lowermost tassel branch, to the nearest 1/2inch
Tassel branch angle (Score 1-3)	TBA	Observe the angle of the central tassel on a scale of 1-3; 1 being an upright central tassel, 2 being a

		horizontal central tassel, and 3 being a drooping central tassel below the horizontal line
Central spike length (in)	CSL	Measure from the top of the central tassel branch to the bottom of the central tassel branch
Pollen shed (Scale 1-9)	PS	Observe the amount of pollen coming from the tassels on a scale of 0-9, 0 being no pollen at all to 9 being heavy pollen shed on all branches of the tassel
Anther color (Score 1-9)	AC	Observe the color of the majority of the anthers on the tassels and score 1-9; 1 being green or yellow, 3 being pink, 5 being red, 7 being dark red, and 9 being purple
Glume color (Score 1-9)	GC	Observe the color of the majority of the glumes on the tassels and score 1-9; 1 being green or yellow, 3 being pink, 5 being red, 7 being dark red, and 9 being purple
Unhusked data		
Silk color (Score 1-9)	SC	Observe the overall color of the silks on an ear and scored1-9; 1 being green or yellow, 3 being pink, 5 being red, 7 being dark red, and 9 being purple
Husk color (Score 1-9)	HC	Observe the overall color of the fresh husk on an ear and score 1-9; 1 being green or yellow, 3 being pink, 5 being red, 7 being dark red, and 9 being purple
Post Harvest Ear Characteristics		
Ear length (in)	EL	Measure from the top of the ear to the bottom of the ear, to the nearest 1/2inch
Ear Circumference (in)	EC	Measure by wrapping a string around the widest part of the ear, marking where the string meets, and measuring that length to the nearest ¼ inch
Ear Diameter (in)	ED	Divide the circumference collected by pi and round to the nearest hundredth
Ear weight (g)	EW	Measure using a digital scale to the nearest hundredth gram
Number of kernel rows	NoKR	Count the rows around the ear
Number of kernels per row	NoKpR	Count the number of kernels up one row from the bottom of the row to the top of the row on an ear
Kernel row distinction (Score 1-2)	KRD	Observe the rows around the ear and determining if the rows were overall distinct from each other and scored a 2, or indistinct from each row and scored a 1
Row pattern (Score 1-3)	RP	Observe the overall pattern on the ear and scoring the ear 1 for a straight patter, 2 for a slightly curved pattern, and 3 for a spiral pattern
Kernel Characteristics		
Kernel length (mm)	KL	Measure from the bottom of the kernel to the top of the kernel, to the nearest millimeter

Kernel width (mm)	KW	Measure at the widest part of the kernel from one side to the other, to the nearest millimeter
Kernel thickness (mm)	KT	Measure by turning the kernel on its side and measuring from one side to the other to the nearest millimeter
Color pattern (Score 1-2)	CP	Observe the overall color of the kernel, determining if the color is all the same and scoring it a 1, or determining if there are segregating colors on the kernel and scoring it a 2

Phenotypic Data Analysis

Phenotypic data for each trait was analyzed for all Pawnee gardens grown from 2017-2022. This analysis was used to determine which Pawnee maize varieties would be used for DNA analysis comparisons. For the protection of these Pawnee varieties, and in accordance with the Pawnee's agreement to study these varieties, the Pawnee maize varieties and grow sites have not been labeled by their specific names or exact locations. Instead have been marked Pawnee variety # for all data in this study and grow site # for anonymity.

Old records of Pawnee maize varieties have been used by the Pawnee people and the PSPS to determine goal characteristics of each variety to breed for. The most thorough and oldest descriptions of these varieties have been written by Will and Hyde (Will and Hyde, 1917). Analysis comparing the maize varieties currently grown by the Pawnee to that of Will and Hyde have been made for each year and each grow site since 2017. For this study, the locations of where these maize varieties were grown were grouped into 11 grow sites total in Nebraska (Figure 4). It was worth noting the proximity of each grow site to the major water sources nearby. The Pawnee had many encampments, villages, and crops grown around the Platte River and Loup River historically.

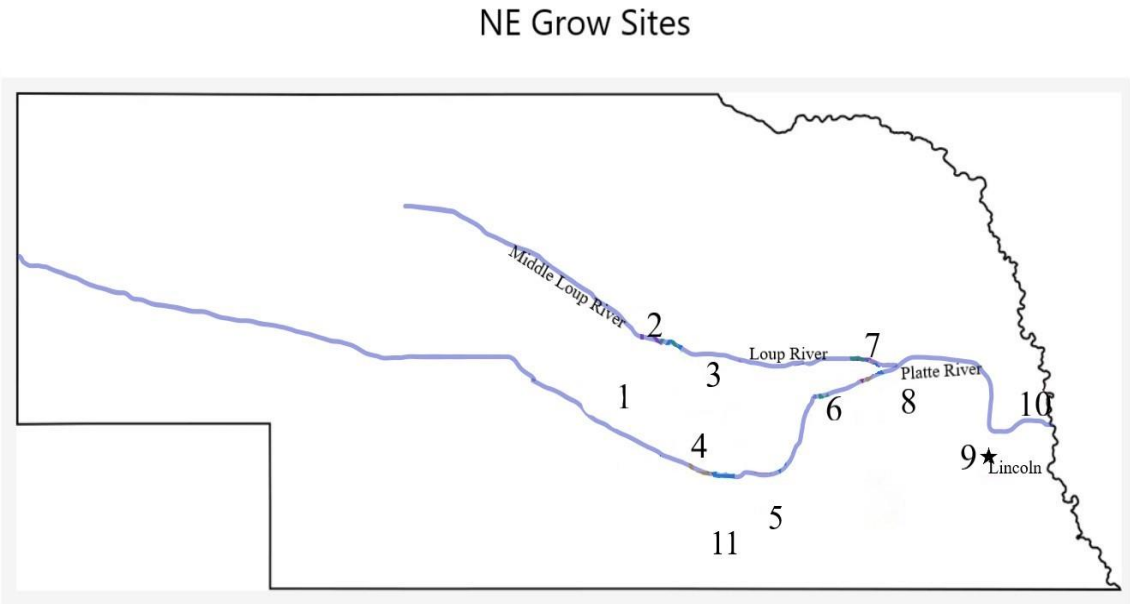


Figure 4. Map of grow sites in Nebraska for PSPS maize from 2017-2022.

Analysis of five Pawnee maize varieties from this study that the PSPS currently grow and also match to a historical description of Pawnee maize varieties was completed in Figures 5-9. From these charts, one could potentially determine where the best grow sites for each trait the PSPS is observing.

From the following analysis, one could infer that Pawnee variety 1 has some traits at grow sites that produce measurements in the ideal range that Will and Hyde recorded, but no traits are exactly as that described by Will and Hyde, shown in Figure 5. For Pawnee variety 1, it looks like grow sites 1 and 8 fall most closely to the recordings of Will and Hyde. Grow sites 1 and 8 are not directly on or within 10miles of a major river, as other grow sites with variety 1. I would recommend growing variety 1 on other sites away from

major river sources to determine the best grow site for this variety.

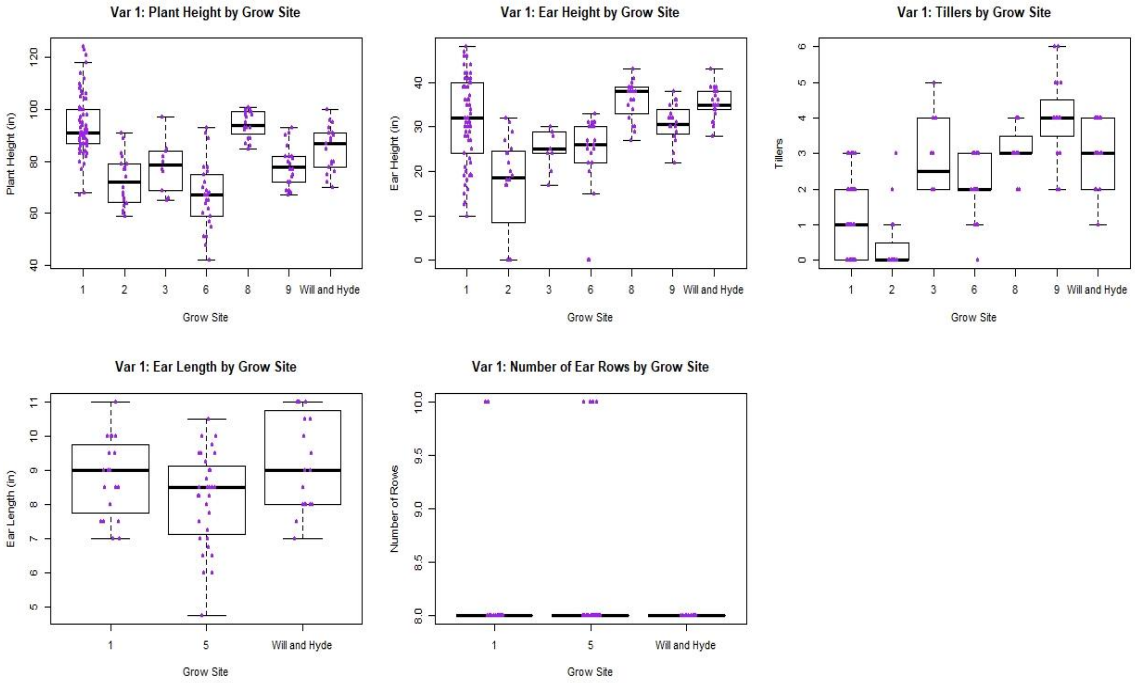


Figure 5 Analysis of Pawnee maize Variety 1 by grow site, compared to Will and Hyde recordings.

From the next analysis, one could derive that both sites 4 and 5 used to grow Pawnee Variety 2 produce results close to that recorded by Will and Hyde, shown in Figure 6. Grow site 4 consistently produced data with closer means to that recorded by Will and Hyde than site 5. Grow site 4 has closer proximity to a major river than that of grow site 5. It would be recommended to grow Pawnee Variety 2 at a site with even closer

proximity to a river than that of grow site 5 and 4 to compare in the future for the PSPS.

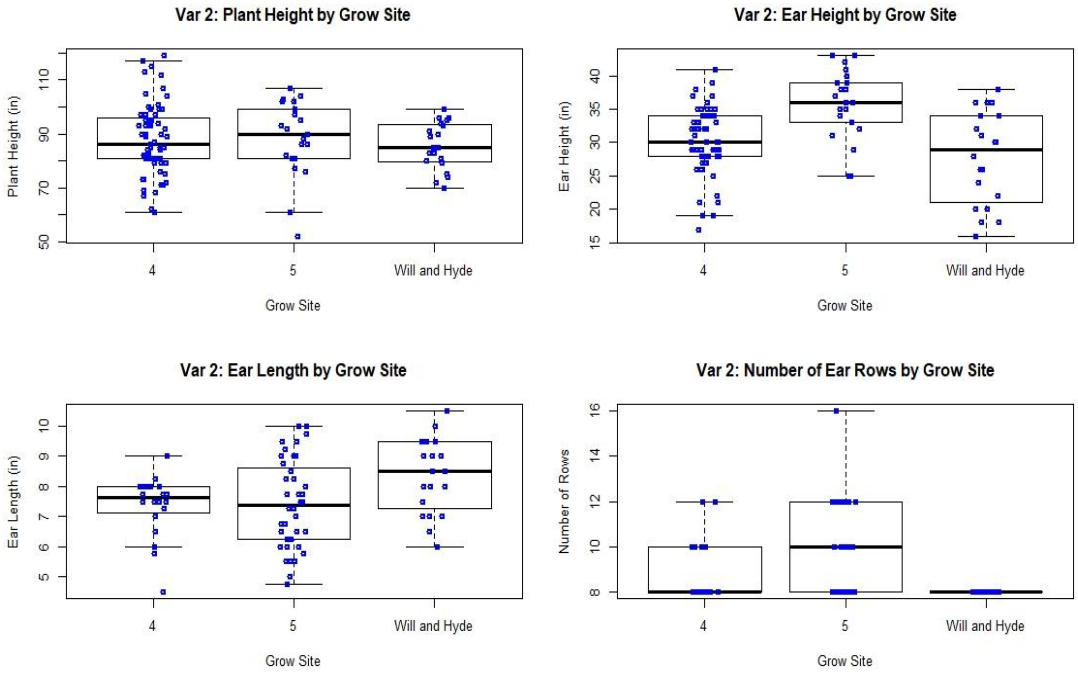


Figure 6 Analysis of Pawnee maize Variety 2 at each grow site, compared to Will and Hyde observations.

From the Variety 4 analysis, one could deduce that both sites 1 and 4 used to grow Pawnee Variety 4 produce results close to that recorded by Will and Hyde, shown in Figure 7. Grow site 1 consistently produced data with closer means to that recorded by Will and Hyde than site 4. Grow site 1 is further in distance to a major river than that of grow site 4. I would recommend growing Pawnee Variety 4 at a site not as close to a

major river in the future for PSPS.

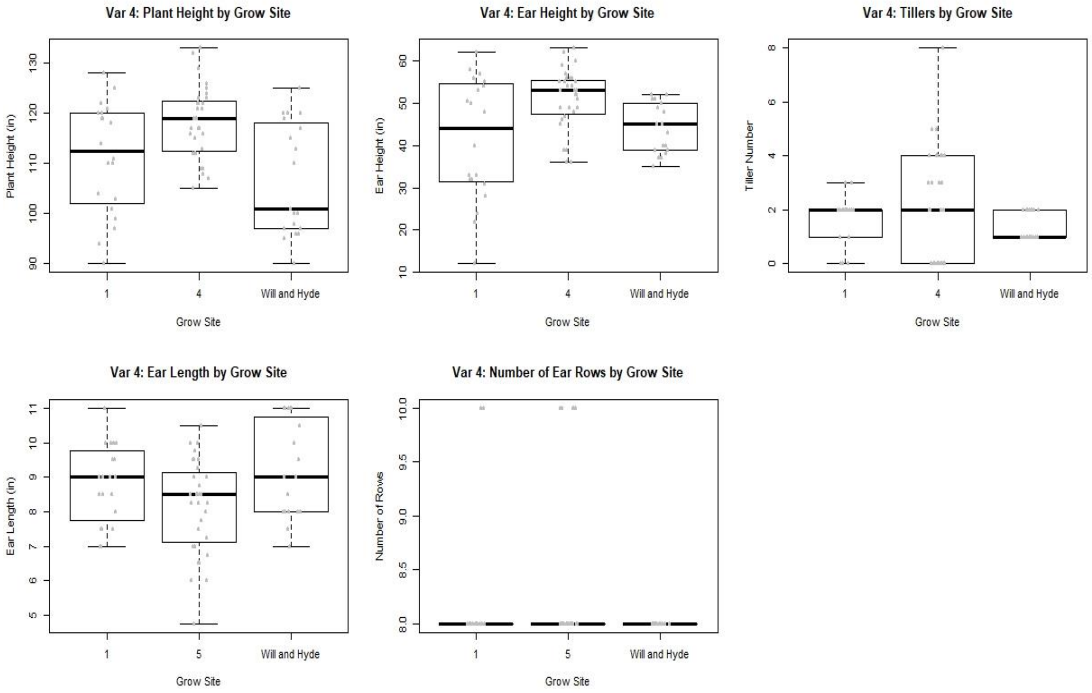


Figure 7 Analysis of Pawnee maize Variety 4 at each grow site, compared to Will and Hyde observations.

From the analysis of Variety 7, one could observe that there has not been an ideal site identified yet to grow Pawnee Variety 7 from those used by PSPS so far, shown in Figure 8. The results produced by the PSPS at each grow site are all over the chart compared to Will and Hyde's observations. For the plant height and ear height, grow site 8 is most closely recorded to that of Will and Hyde's recordings, but there are sites that produce

closer comparisons with the tiller count and ear number count traits than site 8.

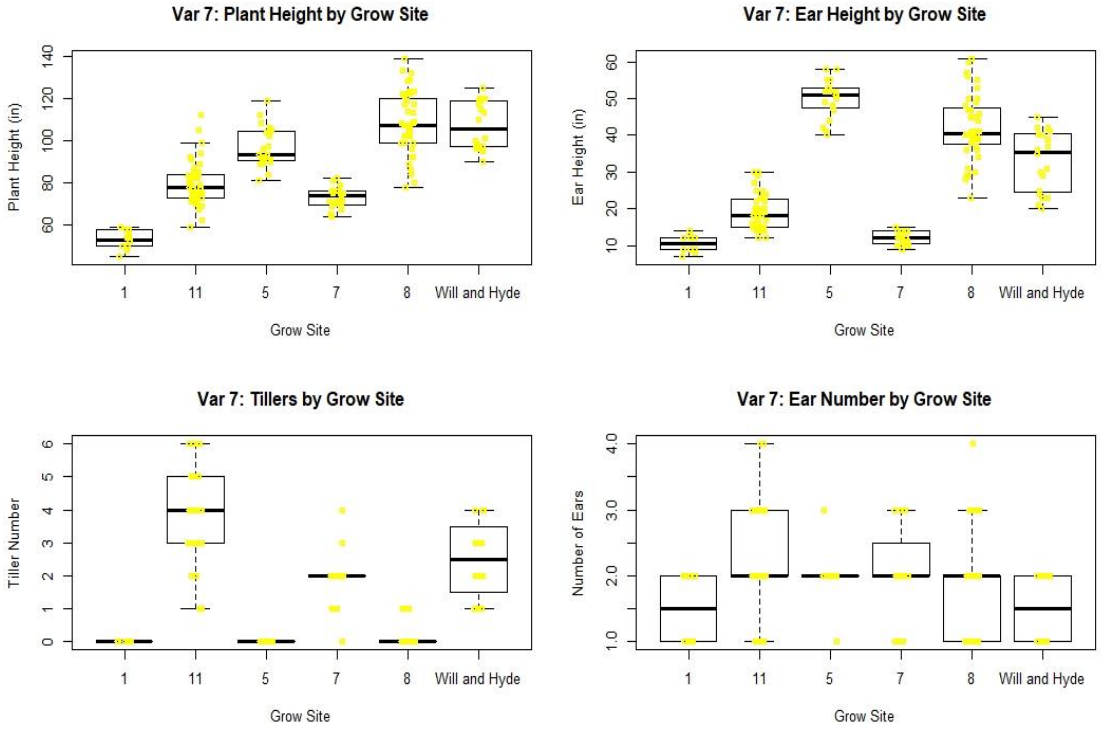


Figure 8 Analysis of Pawnee maize Variety 7 at each grow site, compared to Will and Hyde observations.

Finally, Pawnee Variety 8 has the most traits recorded by the PSPS not close to that of Will and Hyde's recordings, shown in Figure 9. The plant height trait recorded by PSPS does not have a site that produced within Will and Hyde's range of recordings. After analyzing the data for Pawnee Variety 8 grown by PSPS it has been discovered that variety 8 is of a certain type of Pawnee sweet variety maize that is different from the Pawnee sweet variety recorded by Will and Hyde. This may explain why none of the sites used to grow Pawnee variety 8 produced plants within the range that Will and Hyde recorded. It is also worth noting that Pawnee Variety 8 recorded for this study is very

close to the variety Will and Hyde recorded but produce significantly different results.

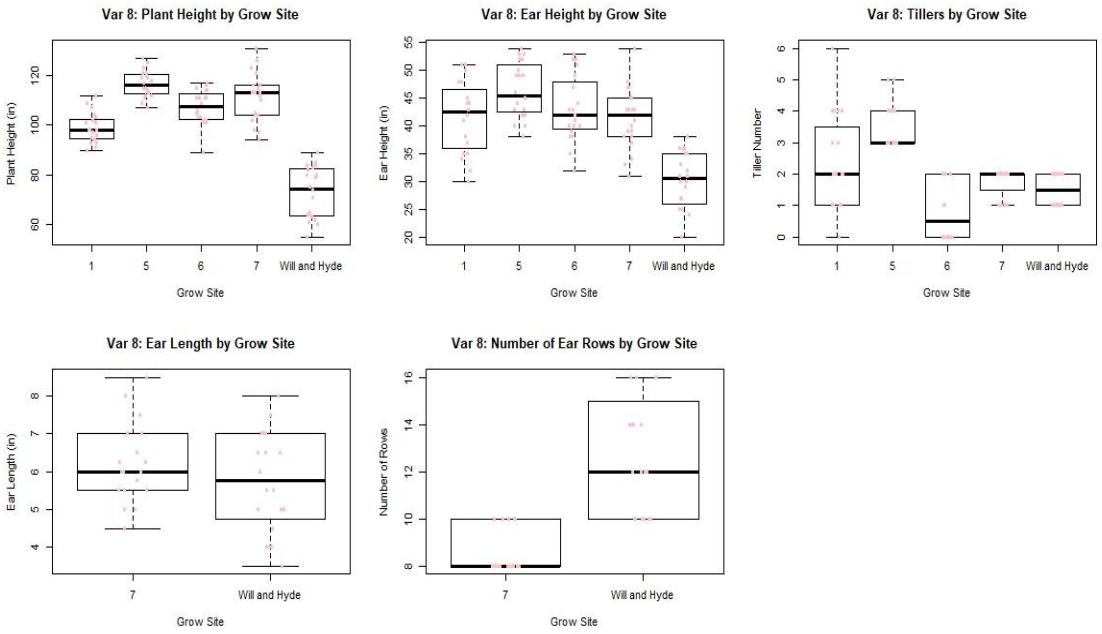


Figure 9 Analysis of Pawnee maize Variety 8 at each grow site, compared to Will and Hyde observations.

The identity issue with Pawnee Variety 8 exposes a concern with older records of Native American crops when used to compare or categorize old Native American crops today. This should be considered when comparing future Pawnee maize varieties grown in the future; the Will and Hyde recordings may not be fully accurate as they were interpreting Pawnee growing techniques after the Pawnee were removed from Nebraska, so their categorization of these varieties may not be fully accurate. This issue is highlighted in a study by Werth in 2007, where Werth explains the importance of landrace maize varieties to be characterized correctly, especially landrace varieties that are old and traditionally grown by Native American (Werth, 2007).

As an example of a complete analysis from the phenotypic data for any given Pawnee variety in this study, Figure 10 illustrates Pawnee Variety 1 comparing plant trait data

across years grown for the PSPS in Nebraska. From the Figure 10 graphs, it can be deduced that plant height for Pawnee Variety 1 has consistently increased in time from 2017 to 2022. This could be caused by environmental variations since the grow site changed for Pawnee Variety 1 each year. This could also be explained by more selective breeding occurring by the Nebraska growers and the PSPS group. Figure 10 also shows that ear height has also increased from 2017 to 2022 but not consistently. The other traits graphed in Figure 10 are more varied throughout the years Pawnee Variety 1 was grown. Again, this is most likely caused by environmental variables as this variety was grown at different sites each year in Nebraska.

Figure 10 represents an example of the analysis that was done for all eight Pawnee varieties for each year traits were recorded from 2017-2022. This analysis can be used to help the PSPS in their breeding efforts to understand and guide their Pawnee maize varieties into the image of their historical Pawnee maize varieties as recorded by historical documents, such as Will and Hyde research.

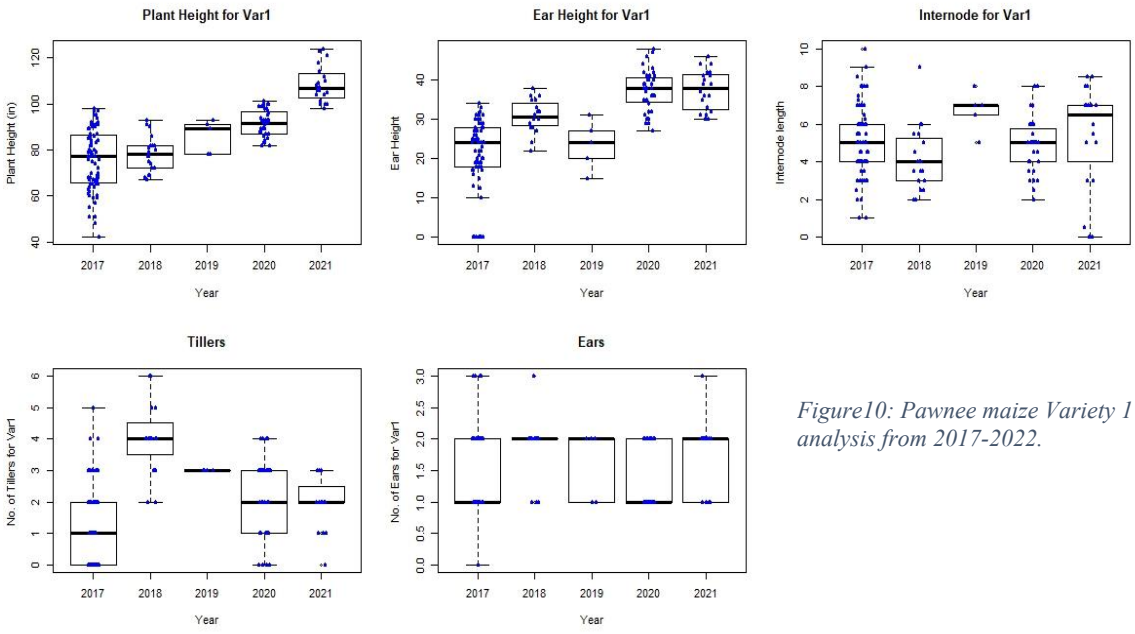


Figure10: Pawnee maize Variety 1 analysis from 2017-2022.

For a complete look at the analysis made for the eight Pawnee varieties observed from 2017-2022 by PSPS, including the five varieties already mentioned in analysis with historical documents, examine Figures 11-12. The charts in Figures 11-12 illustrate a comparison between all eight Pawnee varieties with all data compiled from 2017-2022 by PSPS. Figure 11 depicts all data compiled for plant observations in field and Figure 12 depicts all data compiled for ear and kernel observations post-harvest. From these charts it is clear that these varieties are not of the same type of maize, even though all have been recorded as being typically grown by one group of people in particular, the Pawnee. Many of the characteristics measured are close in the mean shown, but significant differences can be observed as well.

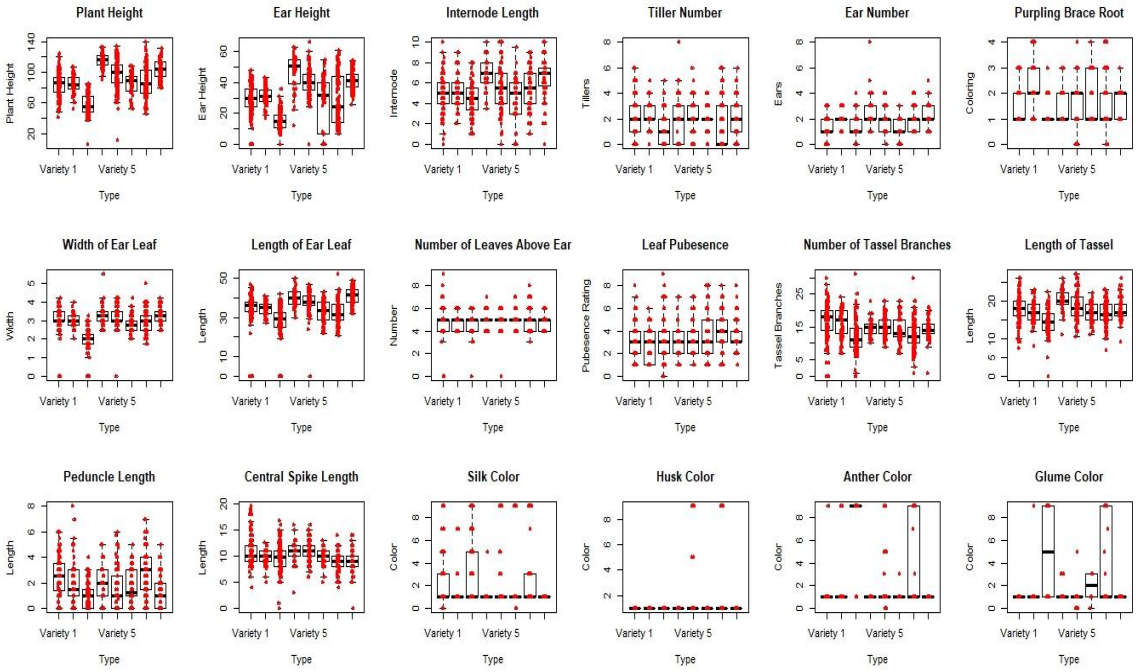


Figure 11: Comparison of all traits recorded for the plant data in field of eight Pawnee maize varieties grown from 2017-2022 by PSPS.

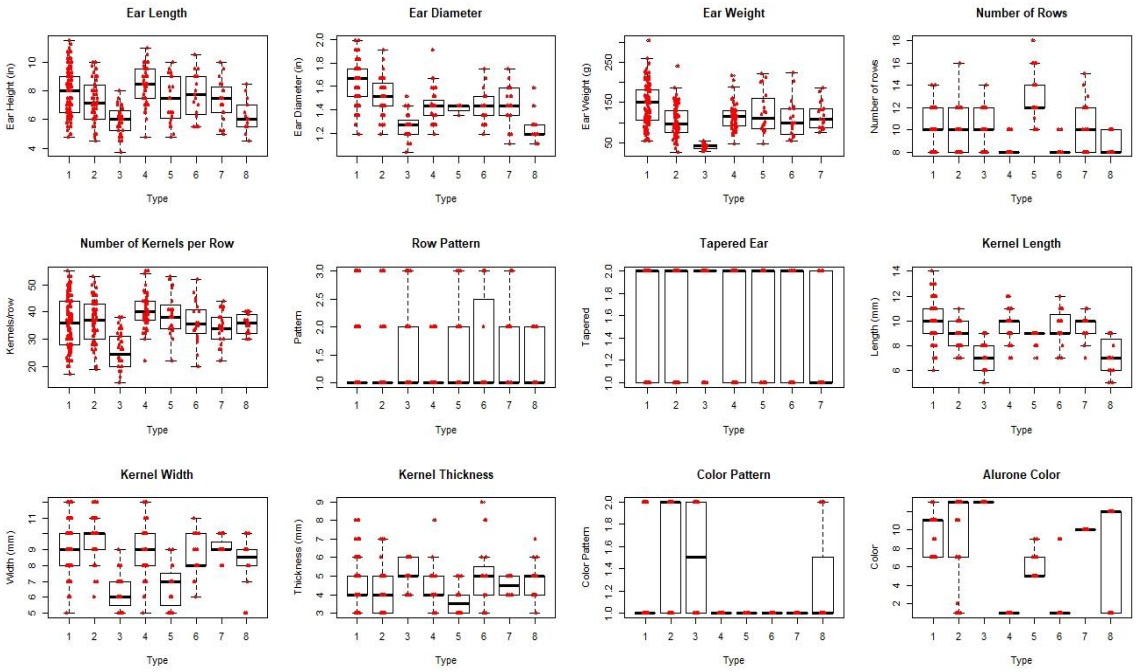


Figure 12: Comparisons of all ear and kernel data collected for eight Pawnee maize varieties from 2017-2022 by PSPS.

Experimental Design for Genotyping

Genetic Resources

This study examined fifteen landraces from the Pawnee tribe verified to have been grown traditionally in Nebraska by the Pawnee from the Pawnee Seed Preservation Society (PSPS). Seed for this study was obtained from PSPS with approval from the Pawnee Nation of Oklahoma. Of these fifteen varieties, eight were landraces grown in Nebraska and observed for data for 6years prior to the writing of this study as mentioned above in phenotypic data. To compare to other types of maize, these landraces were compared genetically with another ongoing study with maize of the sweet variety by Musa Ulutas of the University of Nebraska, Lincoln (UNL). Mr. Ulutas's study included landraces, inbreds, and hybrids to compare the specific Pawnee landrace varieties in this study.

Experimental Design

Ten seeds of each of 21 Pawnee varieties were given by the Pawnee tribe to grow and observe for this study. These Pawnee landrace maize varieties were grown in identical soil in 2in starter cell trays at Pawnee Nation College in Pawnee, Oklahoma (Figure 13). Only 15 varieties grew seedings to observe, and only 4-8 seedings per variety had grown enough for observation in this study. After 7days of growth, the germinated seeds were marked and transported to Lincoln, Nebraska. The young plants remained in a greenhouse at the University of Nebraska in Lincoln, under observation until sample collection.

Figure 13: Pawnee maize varieties grown in 2in starter cell trays in Pawnee, Oklahoma in 2023.

Figure 14: Pawnee maize varieties repotted in 4in pots in Lincoln, Nebraska in 2023.

Plants that were deemed viable for study were separated for sample collection at UNL labs. Plants that were not yet viable for study were repotted in 4inch pots to continue growth in UNL greenhouses for 7 more days (Figure 14). When plants were deemed viable for study, samples were taken from leaf tissues. To ensure an approximately equal amount of leaf tissue samples for DNA extraction for each Pawnee variety, samples were taken from all viable maize plants and dispersed between 6 wells of a cluster plate, then labeled (Table 2).

Table 2: Frequency and location of Pawnee maize varieties leaf tissue samples in cluster plate.

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	Var1	Var1	Var1	Var1	Var1	Var1	Var2	Var2	Var2	Var2	Var2	Var2
B	Var3	Var3	Var3	Var3	Var3	Var3	Var4	Var4	Var4	Var4	Var4	Var4
C	Var5	Var5	Var5	Var5	Var5	Var5	Var6	Var6	Var6	Var6	Var6	Var6
D	Var7	Var7	Var7	Var7	Var7	Var7	Var8	Var8	Var8	Var8	Var8	Var8
E	Var9	Var9	Var9	Var9	Var9	Var9	Var10	Var10	Var10	Var10	Var10	Var10
F	Var11	Var11	Var11	Var11	Var11	Var11	Var12	Var12	Var12	Var12	Var12	Var12
G	Var13	Var13	Var13	Var13	Var13	Var13	Var14	Var14	Var14	Var14	Var14	Var14
H	Var15	Var15	Var15	Var15	Var15	Var15	Empty	Empty	Empty	Empty	Empty	Empty

DNA extraction

Steps for DNA extraction all occurred in UNL labs under controlled conditions. To prepare leaf samples, young fresh leaves were cut from the stalk 15 days after they emerged. Three or four pieces per plant were collected for the test tubes and then transferred to a cluster plate. Leaf tissue from plants of the same variety were combined to fill each test tube in the cluster for that designated variety. Table 2 represents the final

cluster plate alignment, showing the frequency and location for each variety in this study. The samples were then transferred to a freeze drier and dried at -80℃ for five days.

After samples were dried, three to five beads were added to each tube with dried sample material. All tubes were checked again to ensure there were beads with each sample and caps closed. Then samples were placed in a TissueLyser for 2 minutes and turned over halfway through processing time. Once the cycle was over, the racks were knocked so no powder remained in the caps. Caps were then removed from the microtubes.

Figure 15: TissueLyser used in this study to break down leaf tissue samples.

Then, 300µl of RLT Buffer Solution was added to the tissue samples. Samples were then shaken for 1.5 minutes and turned every 45 seconds in the TissueLyser. A centrifuge was used to separate the cell debris from the DNA in the nucleus that we wanted to sequence. Samples were centrifuged for 5 minutes at 6000 rpm.

For the final steps of collecting purified maize DNA, the BioSprint 96 machine was used in the next steps. An S-Block was filled with 300µl of water and labeled. Then 200µl of DNA solution were put in a 96 S-Block and labeled. Next, 200µl of clear plant lysate were transferred into each well of a new S-Block and labeled. Then 200µl of Isopropanol was added to each well in the S-Block. Each sample was placed in the Vortex for 3 minutes. Then the MagAttract solution was added to the same S-Block. Samples were placed in Vortex for 1 minute before subsequent uses.

After the four S-Blocks and two 96 well microplates were prepared, they were then loaded onto the worktable and taken to the BioSprint machine. Each slot of the BioSprint 96 was loaded with the corresponding labeled wells. The BioSprint machine then began the process of mixing the buffers and solutions to collect the final maize DNA samples.

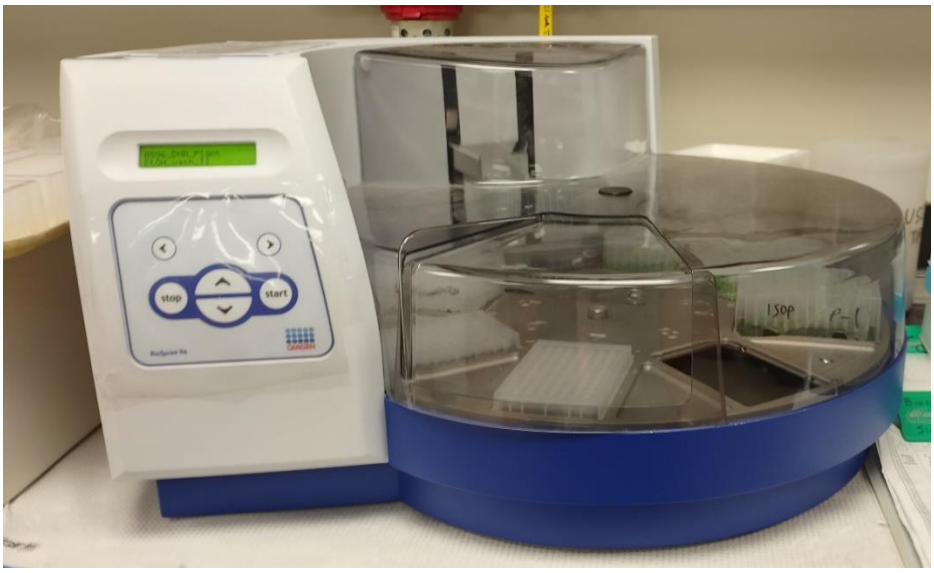


Figure 16: BioSprint96 machine used in this study to complete the final steps of DNA sample collection.

After the cycle was complete, plates and blocks were removed as the display instructed. Used items were discarded according to local safety regulations. The BioSprint was shut off at the power switch. The DNA samples were contained in Plate 6 and moved for testing of quality. Workstations were wiped down and sanitized according to recommended guidelines.

Next the quality of the final DNA samples was tested, first with a simple Spectrophotometer, to measure the concentration of each DNA sample, results in Figure 18. Then we ran 8 random samples of similar concentration through GEL electrophoresis. The 8 samples ran for 10minutes, results shown in Figure 19. Samples were deemed good quality by the lab technician, Musa Ulutas, and advisor, Jinliang Yang. After being tested for quality, the samples were stored in a lab freezer until they were mailed off for sequencing 3 days later. Preparation and mailing of samples were handled by Musa Ulutas.

Figure 17: Spectrophotometer used in this study to determine quality of DNA samples collected.

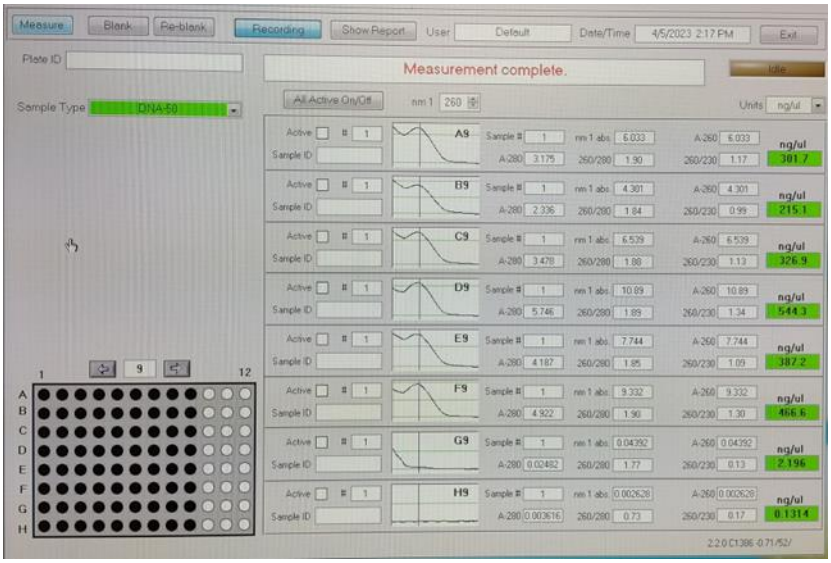


Figure 18: Example of results from spectrophotometer, testing quality of DNA sample collected.

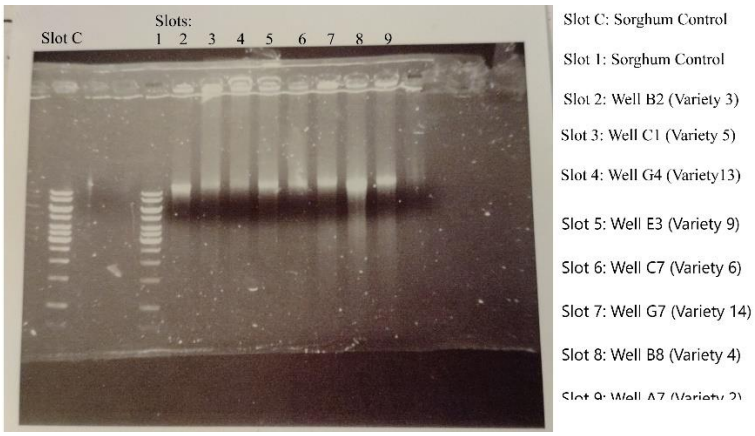


Figure 19: Results from gel electrophoresis, testing quality of DNA samples collected.

DNA Sequencing

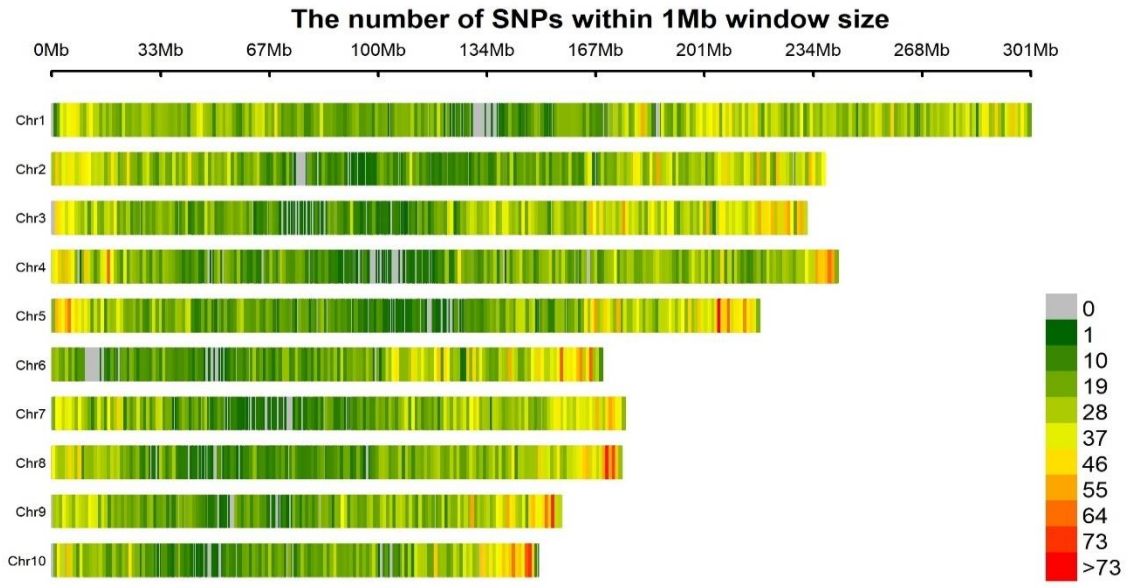
The DNA samples used for this study were mailed to a lab outside of UNL for DNA sequencing along with several hundred samples of sweet variety maize in a similar DNA study conducted by Musa Ulutas at UNL. Those sweet variety maize samples were used to compare with the Pawnee maize varieties.

Genotyping by target sequencing, or GBTS, was used because of its relatively inexpensive cost and quality compared to other target sequencing techniques (Guo et al, 2021). GBTS examines the variations within the DNA, called single nucleotide polymorphisms, or SNPs. We then could compare these SNPS to each other from the Pawnee landrace varieties and with the batch of sweet corn. The more SNPs found, the more differences genetically between the varieties.

Statistical Analysis and Results

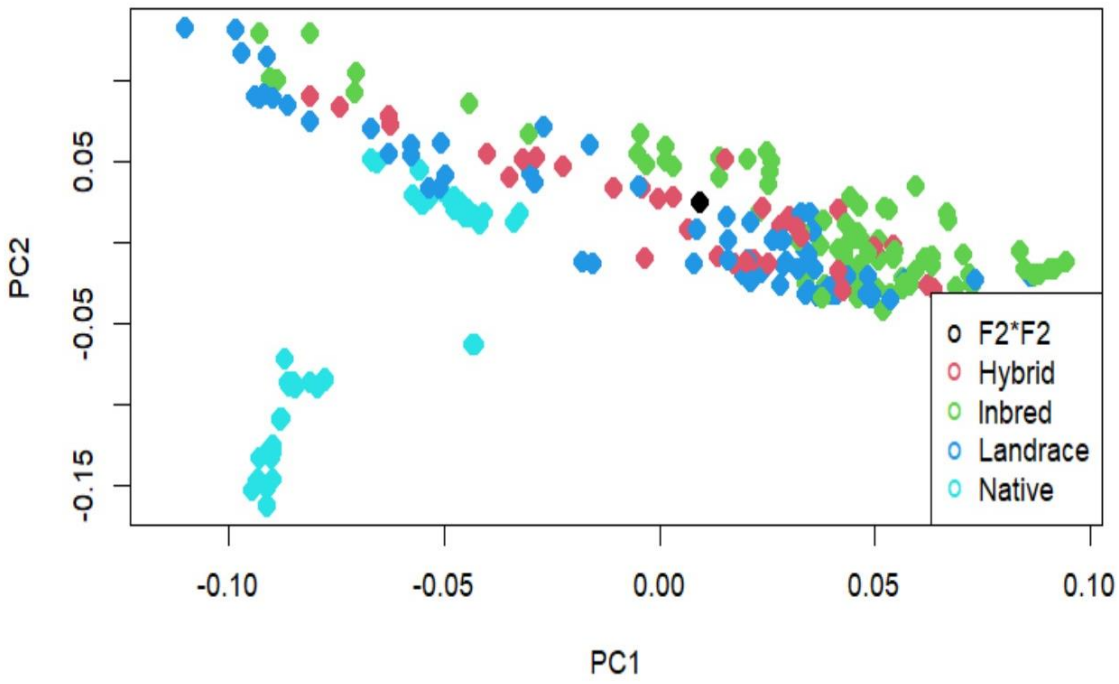
After the DNA results were received, statistical analysis began comparing the 15 Pawnee landrace varieties along with the sweet variety maize. The first analysis was to test the quality of the results by calculating the overall differences among varieties sequenced at the targeted regions. SNPs were discovered across the 10 maize chromosomes, with a SNP density reaching up to 70 SNPs per megabase(Mb), as shown in Figure 20.

Figure 20: Results of quality analysis of DNA samples, indicating the number of SNPs detected.



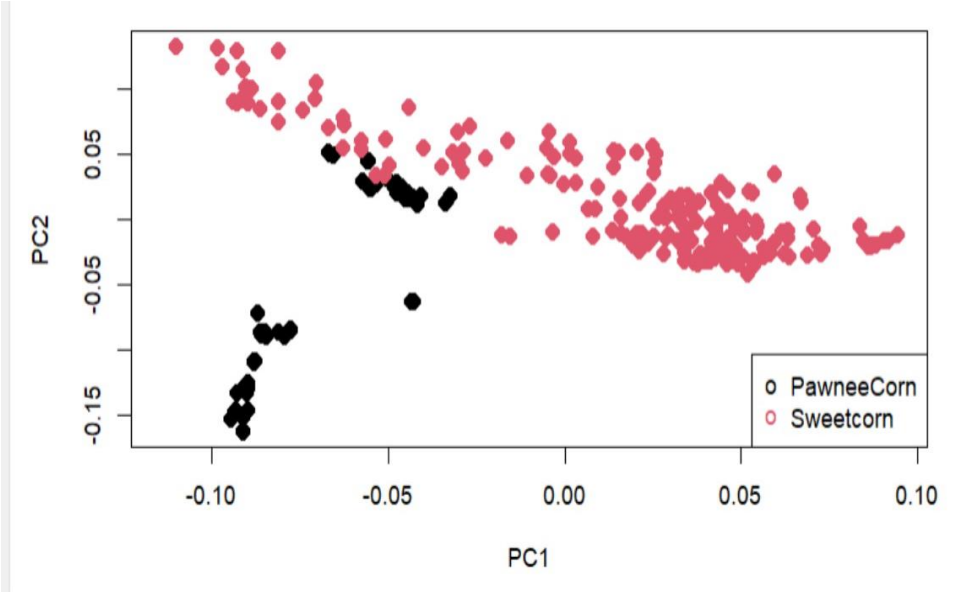
The next set of results calculated comparing the genetics of the sweet maize grouped in this DNA sequencing to that of the 15 Pawnee maize varieties. Those results with the modern sweet maize varieties grouped in their categories according to their lineage are seen in Figure 21. This graph is added to show the comparison of the landrace Pawnee varieties with the landrace modern sweet varieties. Some of the Pawnee varieties do end up close to the modern sweet varieties genetically, especially with the modern landraces.

Figure 21: Principal component analysis (PCA) using maize materials sequenced as part of this research.



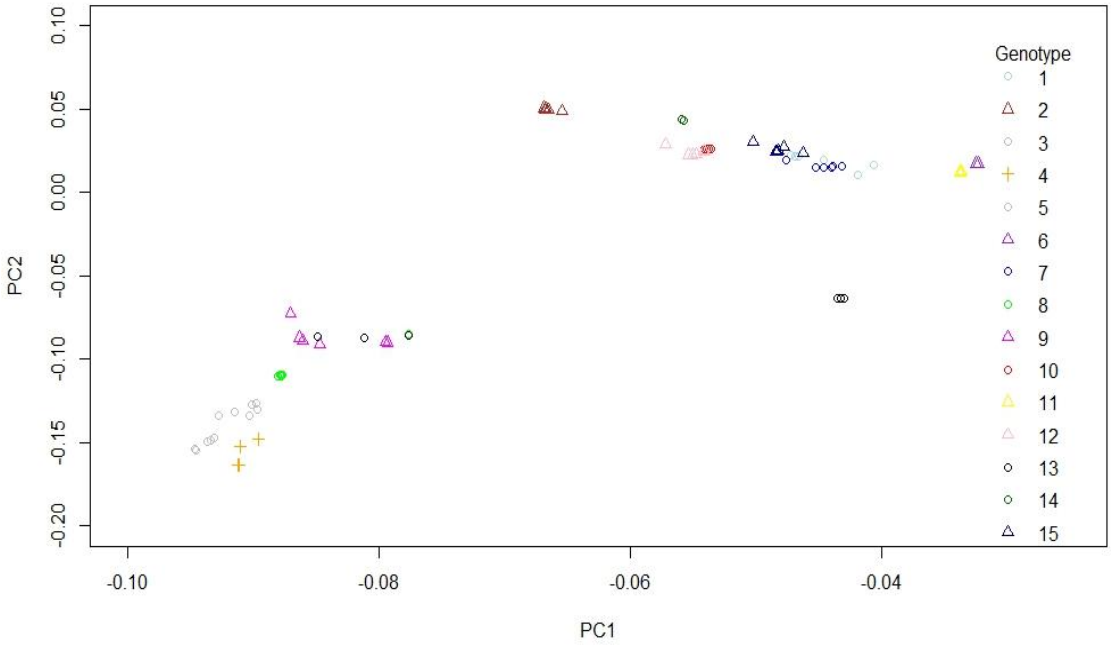
For a broader view showcasing the Pawnee varieties more clearly compared to the modern sweet maize varieties, Figure 22 was created. This figure clearly illustrates how some of the Pawnee varieties are outside the realm of the modern sweet varieties and some Pawnee varieties are clearly genetically similar to modern sweet varieties.

Figure 22: Graph illustrating genetic difference between modern sweet corn and Pawnee corn varieties from this study.



For a closer look at the fifteen Pawnee varieties in this study, Figure 23 illustrates the comparison of just those Pawnee varieties. Here it is clearer that these Pawnee varieties are in fact different from each other genetically, even though grown in the same way by one people. Although it is known that one of the fifteen Pawnee varieties is a sweet variety, there are several Pawnee varieties that are grouped with the modern sweet varieties as seen in Figures 21 and 22. From Figure 23, it is also worth noting the one variety separated from the bigger two groupings. Pawnee variety 7 stands out in Figure 23, which is a variety documented for its phenotypic traits. Further genetic analysis and future research in this variety would be a priority in the continuation of this research.

Figure 23: Graph illustrating the genetic differences among the 15 Pawnee landrace varieties in this study.



Discussion

The graphs of Figures 21-23 show the clusters of 15 Pawnee varieties that indicate there are differences genetically among the Pawnee varieties from each other and from the sweet corn sequenced as well. Further analysis needs to be completed to consider all the genetic differences among the Pawnee landrace varieties.

For a closer look at these genetic differences, first I considered comparing different seeds for a given variety. Figure 24 used the principal component analysis, as shown in Figure 21-23, but only for data collected on one Pawnee maize variety, genotype 1. This graph shows slight differences among the samples' DNA. The 6 samples collected from this one Pawnee maize variety could be showing slightly different from each other because the seeds used in this study were collected from different years and different grow sites but were all of the same Pawnee maize variety. There may also be some human errors in sample collection with cross contamination at some point in leaf tissue collection or in DNA retrieval.

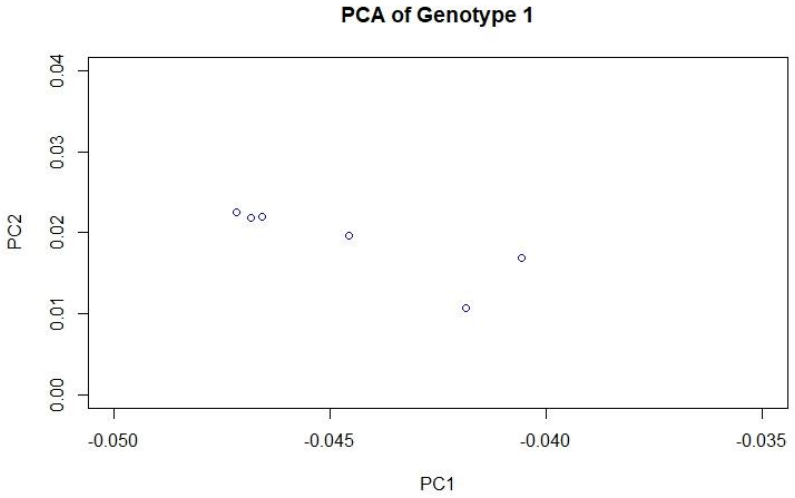


Figure 24: Principal component analysis of only one genotype of Pawnee maize.

To continue examining genetic differences among Pawnee maize varieties, I compared three different types of Pawnee maize. From documentation with the PSPS, I know the varieties in this comparison are each different in their classification as either a flour type maize, a flint type maize, and a sweet type of maize. Figure 25 shows the analysis of Pawnee maize genotype 1, genotype 2, and genotype 4. From this graph, it is clear that these Pawnee maize varieties are different from each other, forming in 3 distinct clusters from each other.

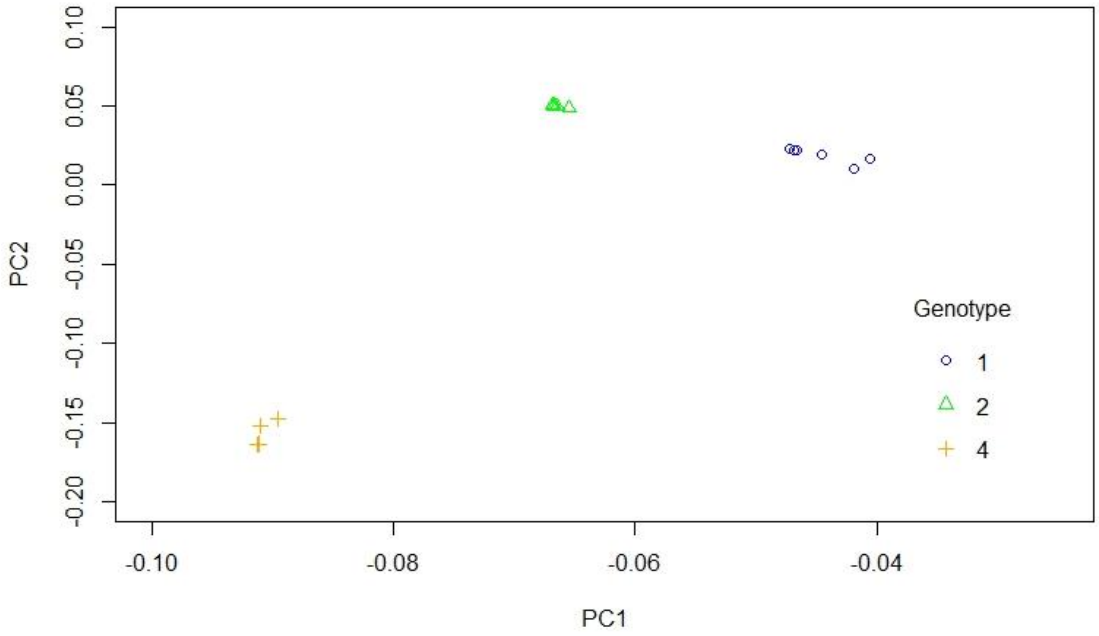


Figure 25: PCA comparing 3 Pawnee maize varieties of different types to indicate how significant their differences are genetically.

Lastly, I compared the three previous Pawnee genotypes with that of the modern sweet varieties. Figure 26 shows that analysis comparing Pawnee genotype 1, 2, and 4, with all the modern sweet corn that was also analyzed in this study. From this graph, two of the Pawnee genotype clusters are grouped closely to the modern sweet maize clustering. Genotypes 1 and 2 could be closely related to some of the sweet maize varieties analyzed

in this study. Genotype 4 remains in its own grouping significantly different from all other genotypes graphed here. I would recommend placing the Pawnee maize variety associated with genotype 4 as a priority for future study.

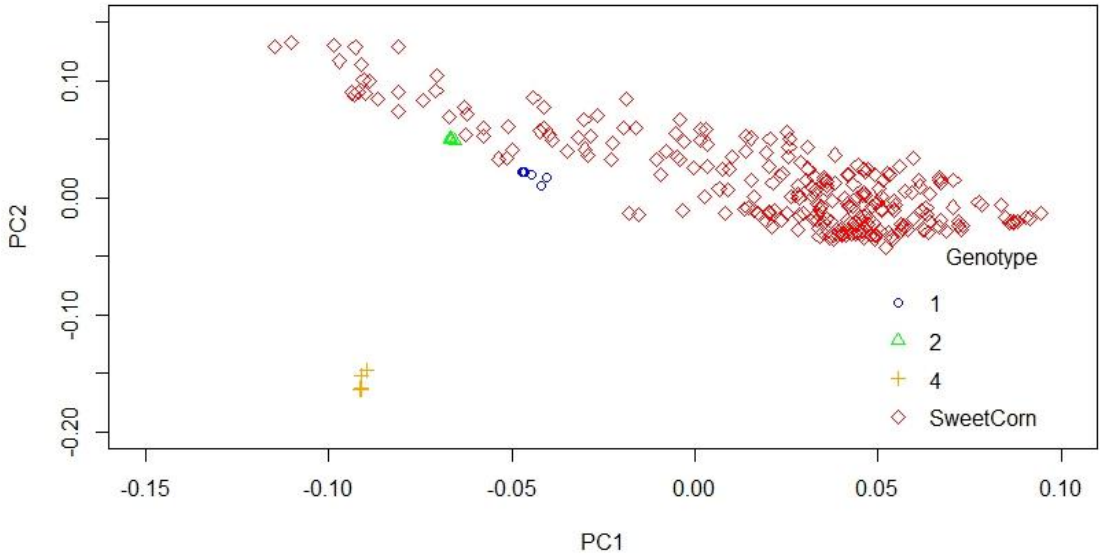


Figure 26: PCA of Pawnee maize genotypes 1,2, and 4 compared next to modern sweet varieties analyzed in this study.

These results are an important first step in categorizing Pawnee landrace varieties. Although we can claim that these varieties historically and presently grown by the Pawnee people are indeed different from each other genetically, more analyses need to be completed.

Considerations and Recommendations

When comparing Native American landrace varieties in a research study, there is a considerable amount of trust and discretion to be had with the owners of these varieties and the data being collected on these varieties. This study was conducted with the approval of the Pawnee Nation, the Pawnee Seed Preservation Society, and the University

of Nebraska; with every step monitored by a representative of one of these groups present at each major step in the experiment. With that, more trust and full disclosure between these groups would improve the quality of this experiment.

I would recommend that if a future study was allowed on the Pawnee landrace varieties of maize, that the study be completed at one trusted location from start to completion with allowances of more seeds when there is no germination from some Pawnee maize varieties. Also, an allowance to grow and breed several generations of Pawnee maize varieties would help to ensure quality of DNA samples by controlling the conditions each generation was grown and knowing the provenance of each generation. More analysis on heritability could also be completed for each trait measured under these conditions.

Conclusions

This research sets a beginning comparative baseline for the Pawnee landrace maize varieties on which research can continue to grow our understanding of these maize varieties. Furthermore, this study begins the process of genetically classifying Pawnee maize varieties through SNP comparisons using GBTS based genotyping. Further genetic research is needed to better understand and classify these Pawnee maize varieties.

To conclude, the study and research of landrace varieties is important not only to the agricultural community and the research community, but also to the growers of landrace varieties and their cultures. This study was a look into just one group of landrace maize varieties from one tribe. This study is just a beginning of more in-depth research into Indigenous landrace varieties and their genetics. There is a lot to do to move forward, but the past must be sorted first. Studies of the past conducted on Indigenous peoples crops and agricultural practices is not complete, especially if the study was conducted by a non-member of that Indigenous group. Knowledge gets lost in translation, products and produce get mis-categorized leading to the taking of Indigenous property for profits and gains. Trust and discretion must be the biggest take-aways from this study while looking forward toward continuing research in Indigenous crops.

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