MAIZE *SUGARY ENHANCER1 (SE1)* IS A PRESENCE-ABSENCE VARIANT OF A PREVIOUSLY UNCHARACTERIZED GENE
AND
DEVELOPMENT OF EDUCATIONAL VIDEOS TO RAISE THE PROFILE OF PLANT BREEDING AND IMPROVE CURRICULA

by

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DEVELOPMENT OF EDUCATIONAL VIDEOS TO RAISE THE PROFILE OF PLANT BREEDING AND IMPROVE CURRICULA

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Under the supervision of Professor Shawn M. Kaeppler

At the University of Wisconsin-Madison

Carbohydrate metabolism is a biologically, economically, and culturally important process in crop plants. Humans have selected many crop species such as maize (*Zea mays L.*) in ways that have resulted in changes to carbohydrate metabolic pathways for different end uses, such as in grain for food and livestock, sweet corn for eating fresh, and in many crops for biofuel production. Understanding the underlying genetics of this pathway and the similarities and differences across species is therefore exceedingly important.

A previously uncharacterized starch metabolic pathway mutant, *sugary enhancer1 (se1)*, is a recessive modifier of *sugary1 (su1)* sweet corn that increases the sugar content while maintaining an appealing creamy texture. This allele has been incorporated into many sweet corn varieties since its discovery in the 1970s, however, breeding varieties with this trait has been difficult due to the fact that there has not been a clear codominant marker to test for the presence and absence of *se1*.

A genetic stock was developed that allowed the presence of *se1* to be visually scored in segregating ears, which were used to genetically map *se1* to a single gene model located on the distal end of the long arm of chromosome 2. The *se1* allele is comprised of a 637 bp deletion that encompasses all of annotated gene model AC217415.3_FG004. An analysis of homology found that this gene is specific to monocots, and an expression analysis in B73 showed that the gene is

expressed in the endosperm, embryo, and developing leaf. The *se1* allele increased water soluble polysaccharide (WSP) and decreased amylopectin in maize endosperm, but there was no overall effect on starch content in mature leaves due to *se1*. This discovery will lead to a greater understanding of starch metabolism, the marker developed will assist in breeding, and a potential new starch pathway mutant was discovered which could provide the foundation for future scientific discoveries.

There is a present need for increased training for plant breeders to meet the growing needs of the human population. Plant breeding is an obscure yet vitally essential field, so to raise the profile of plant breeding among young students, a series of videos called *Fields of Study* was developed. These feature interviews with plant breeders who talk about what they do as plant breeders, what they love about their chosen profession, and what educational backgrounds can prepare students for pursuing plant breeding as a career. To help broaden the education of students in college biology courses, assist with the training of plant breeders, and educate hobbyist breeders, a second video series, *Pollination Methods* was developed. Each video focuses on one or two major crops, their genetics, and shows how to make controlled crosses with these plants. Both video series have been well received, and have already made contributions to the recruitment and training of future plant breeders.

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Director in a nonprofit organization we started, and a co-blogger in crime, but also as a good friend. More than a decade after I took Pam's class at UC Davis, she has continued to be a great mentor, and has given me so many opportunities to become involved in the intersections of science and society. Together, you helped me to realize my dream of being a scientist-communicator.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Chapter 2 Page

LIST OF FIGURES

Chapter 2 Page

Chapter 3 Page

LIST OF APPENDICES

CHAPTER 1: Literature Review

1.1 The maize (*Zea mays L.*) kernel

The maize (*Zea mays L.*) kernel is a caryopsis, which is a one-seeded fruit containing an embryo, endosperm, and remnants of the seed coats and nucellus inside a pericarp, which functions as a seed coat in cereal crops (True et al., 1893). The kernel develops from a single ovary containing one ovule and typically only one pollination event (Kiesselbach, 1949).

The maize plant and its ancestor, Teosinte (*Zea mays L. ssp. parviglumis*) are typically monoecious, with separate male and female flowers. The male (staminate) flowers develop at the apical meristem of the plant in a branched structure called the tassel. The female (pistillate) flowers are found on the ears, which develop from shoots that branch off of the main stem of the plant at one or more nodes. The ear, which evolved during domestication from a small rachis that produces 5-10 seeds, today contains 500-1200 ovaries that can develop into kernels after pollination. Each ovule consists of an embryo sac that contains one ovum, several adjacent synergids and antipodal cells, and two polar nuclei (Kiesselbach, 1949).

During the process of pollination, a trinucleate pollen grain shed by anthers in the tassel lands and germinates on the silk (an elongated stigma), forming a pollen tube that grows down the length of the silk to a single ovary. One of the three nuclei forms the pollen tube, while the remaining two are the sperm cells which enter the embryo sac in a process called double fertilization (Guignard, 1899; Strasburger, 1900). In this process, one sperm nucleus fuses with the ovum to form the diploid (2n=20) embryo, while the second nucleus fuses with the two polar nuclei to form a triploid (3n=30) tissue that develops into the endosperm (Kiesselbach, 1949). Typically, the sperm cell nuclei both originate from the same pollen tube, however in some cases pollen tubes from separate pollen grains can fuse with the ovum and polar nuclei respectively,

which is known as hetero-fertilization (Sprauge, 1932). While the ova and pollen are the gametophyte, and the parent plant and embryo are sporophytes, the endosperm is considered neither sporophytic nor gametophytic (Esau, 1977; Raven et al., 1992).

1.2 The origin and development of cereal endosperm

The endosperm is a tissue common to angiosperm plants, and is particularly important for cereal crops as it serves as a source of nourishment for the developing embryo and the germinating seed (Strasburger, 1879). It is formed as a result of a double-fertilization event, with one sperm nucleus (1n) fusing with two polar nuclei (2n) to form a triploid (3n) tissue. Due to the unique characteristics of its development, there has been some debate as to its origin. There are two competing origin models, one is that the endosperm is an extension of the female gametophyte with an additional genome that adds to the process, and the second is that the endosperm is a supernumary embryo that has evolved to instead support the normal embryo (Friedman, 2001). Friedman et al. (1998) determined that *Ephedra trifurca*, a close relative of angiosperms, produces a second supernumary embryo through double-fertilization that does not survive to produce a seed, yet also does not nourish the normal embryo as does the endosperm in angiosperms. This suggests that the second hypothesis is more likely to be accurate. It is also interesting to note that many genes are often expressed in both endosperm and embryo alike (Lopes and Larkins, 1993), which could suggest a common origin of the two tissues.

Endosperm development in maize and other cereals follows three distinct stages: early development, differentiation, and maturation (Bosnes et al., 1992). Following the double-fertilization event in early development, the endosperm forms a syncitium through rapid replication of the nuclei without cell division. In the first three days after pollination (DAP), up

to 512 nuclei may be produced in this multinucleate cell, which is notably faster than replication in the developing embryo (Sabelli and Larkins, 2009). At this point, cellularization begins with the formation of radial microtubule systems that extend from the membranes of each nucleus in the syncitium from the periphery toward the center. Cell membranes and cell walls are formed separating each individual nucleus, and mitosis continues between 4 and 10 DAP (Sabelli et al., 2007; Olsen, 2001).

After cellularization and mitosis completes, the endosperm enters the differentiation stage which is characterized by endoreduplication at 10 to 18 DAP, and cellular differentiation into four tissue types, from 7 to 12 DAP. During endoreduplication, endosperm cells undergo continual rounds of DNA replication without cell division, producing cells with a genome number of 100N or more (Schweizer et al., 1995). This process coincides with an increase in starch and storage protein production in endosperm cells. Cells at the base of the endosperm differentiate into transfer cells, which facilitate the transport of nutrients from the parent plant to the other cells in the endosperm. Several cell layers that surround the developing embryo differentiate into the embryo-surrounding region (ESR) cells, which may help nourish and exchange signals with the embryo (Cossegal et al., 2007). Cells in the outer layer differentiate into the aleurone, which surrounds the endosperm except for the transfer cells and the ESR cells, and is important for producing hydrolytic enzymes to digest starch for the germinating seed (Sabelli and Larkins, 2009). Finally, cells in the center of the endosperm differentiate into starchy endosperm cells which accumulate starch and storage proteins from 14 to 28 DAP (Olsen, 2001).

The final maturation stage of endosperm development is characterized by programmed cell death and desiccation of the endosperm cells, and begins at approximately 16 DAP in the

central endosperm cells as well as the apical cells at the top of the kernel (Young and Gallie, 2000). During this process, nucleases and proteases degrade nuclear DNA under the influence of gibberellic acid, ethylene, and abscisic acid. By 40 DAP, the endosperm cells have completely undergone apoptosis and dessication except for the aleurone layer which remains alive but dormant until rehydrated during germination (Berger, 1999; Sabelli and Larkins, 2009).

1.3 Starch metabolism and storage in cereal endosperm

The production of starch in cereal endosperm is a complex process that occurs by means of a multi-component pathway that is still being elucidated. Research in maize and rice (*Oryza sativa*) has been particularly important in determining the steps in this pathway (Preiss et al., 1991; Nelson and Pan, 1995; Hannah, 2005; Jeon et al., 2010). Starch consists of two homopolymers of glucose, amylose and amylopectin. Amylose is a linear polymer of glucose subunits joined by α-1,4 linkages, with a small number of α-1,6 linkages on approximately 1 out of every 1,000 residues. In maize, amylose makes up approximately 20-36% of the weight of the starch granule (Deatherage et al., 1955), with amylopectin making up the remainder. Amylopectin is a highly organized, branched polymer of glucose subunits joined by α-1,4 linkages with the addition of α-1,6 linked branches of 12-20 glucose polymers in length and arranged in clusters every 9 nm (Smith 2001).

Figure 1.1 shows the essential steps that lead to starch synthesis in the cells of sink tissues such as endosperm (from Spielbauer et al., 2006). Sucrose is imported into the cell and either hydrolyzed into glucose and fructose by invertase, or converted into fructose and uracil-diphospate glucose (UDP glucose) by sucrose synthase (Chourey and Nelson, 1976). These monosaccharides are then converted into hexose phosphates, which can be transported into the

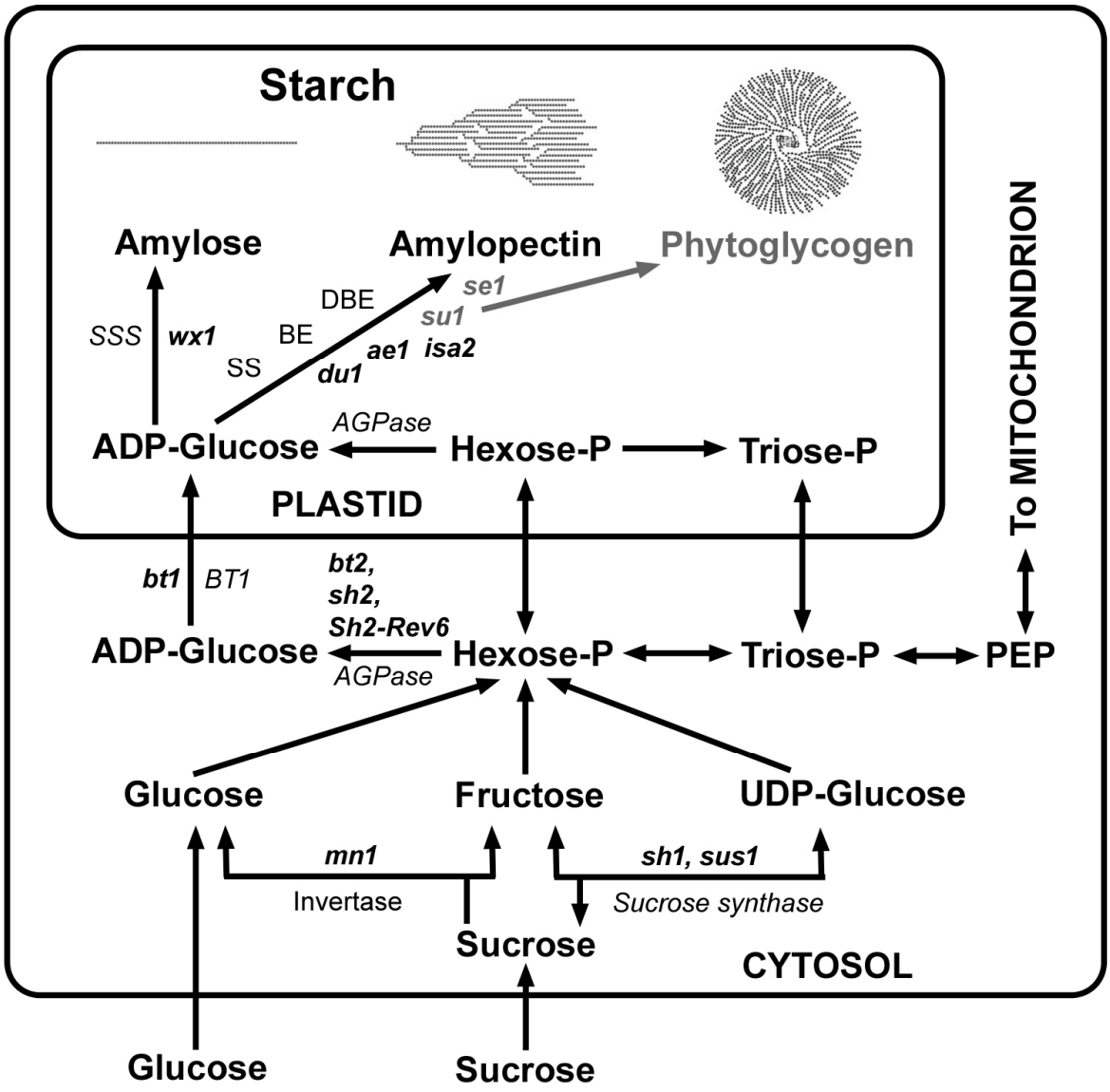
amyloplast before or after being converted into adenosine diphosphate glucose (ADP-glucose) by ADP glucose pyrophosphorylase (AGPase). This conversion into ADP glucose is the first committed step in starch synthesis. ADP glucose in the amyloplast is synthesized into starch by a combination of several starch synthases (SS), branching enzymes (BE), and debranching enzymes (DBE). Forward genetics approaches have identified many of the genes involved in the maize carbohydrate pathway.

The recessive mutant *miniature seed1 (mn1)* reduces the quantity of hexose sugars that are transported into the cell, resulting in a diminutive seed phenotype (Vilhar et al., 2002), and MN1 has been identified as an Invertase (Cheng et al. 1996). Maize has two sucrose synthase homologs, *Shrunken1 (Sh1)* and *Sucrose synthase1 (Sus1)*, which differ in that *Sh1* is the main form of the enzyme (Hannah, 1997), and the loss of which causes a severely shrunken kernel, whereas *sus1* recessive mutants have no apparent phenotypic alterations from wild-type (Chourey and Taliercio, 1994).

The next step, once sucrose has been converted into glucose in the cytosol, is conversion into ADP-glucose by ADP glucose pyrophosphorylase (AGPase). Although this reaction is reversible, the forward direction is highly energetically favored, so it is termed the first committed step in starch synthesis (Mitsui et al., 2010). AGPase is a heterotetrameric enzyme composed of two large and two small subunits. The large subunit is encoded by *Shrunken2 (Sh2)*, and the small subunit is encoded by *Brittle2 (Bt2)*, and are highly similar on a sequence level (Bhave et al., 1990; Bae et al., 1990). In mature kernels, both the *sh2* and *bt2* mutants increase the wild-type sucrose content of 1.5% by weight to approximately 15%, and decrease starch content from approximately 65% to 25% and 35%, respectively (Cameron and Teas, 1954; Laughnan, 1953; Teas and Teas, 1953), producing shrunken, opaque kernels. The increase in

sugar caused by the *sh2* mutant has given rise to many successful "supersweet" sweet corn varieties with double the sugar content of sugary sweet corn (Tracy, 2001).

Figure 1.1 Pathways of the central carbohydrate metabolism in developing maize kernels.



The map is based on Spielbauer et al. 2006, and recent literature on maize biochemistry. Starch-deficient mutants are shown in boldface type next to the affected reaction step. *ae1*, *amylose extender1*; *bt1*, *brittle1*; *bt2*, *brittle2*; *mn1*, *miniature1*; *sh1*, *shrunken1*; *sh2*, *shrunken2*; *su1*, *sugary1*; *sus1*, *sucrose synthase1*; *wx1*, *waxy1*; *AGPase*; ADP-glucose pyrophosphorylase; DBE, starch debranching enzyme; BE, starch branching enzyme; SS, soluble starch synthase; and SS, granule-bound starch synthase.

ADP glucose is transported into the amyloplast by an adenylate translocator encoded by *Brittle1 (Bt1)*, and the loss of BT1 activity in homozygous *bt1* genotypes produces a phenotype similar to *sh2* and *bt2* kernels (Cao et al., 1995 Sullivan et al., 1991; Sullivan and Kaneko, 1995). Once in the amyloplast, starch synthases (SS) join glucose monomers together with a α-1,4 linkages by attaching the glucose from ADP glucose to the non-reducing end of an existing glucose chain, releasing an ADP molecule (Myers et al, 2000). Five classes of SS enzyme have been described, including granule-bound SS (GBSS), SSI, SSII, SSIII, and SSIV. GBSS is involved in the sysnthesis of amylose, and the loss of GBSS activity in homozygous *waxy1 (wx1)* mutants results in the loss of amylose synthesis (Cao et al., 2000; Denyer, 2001; Sprague et al., 1946).

The remaining SS enzymes are believed to only function in the synthesis of amylopectin. The current model for the function of SS enzymes (James and Myers, 2009) describes them as functioning on amylopectin chains of increasing lengths, with SSI acting on the shortest chains (less than 10 subunits), SSII on chains of 12-25 subunits in the branch clusters, and SSIII on chains longer than 30 subunits. In maize, only SSIIa and SSIII genes have been identified with mutant alleles. The SSIIa isoform in maize is encoded by the *Sugary2* (*Su2*) locus, and the SSIII isoform is encoded by *Dull1* (*Du1*). Homozygous *dull1* mutants produce a tarnished kernel phenotype (Cao et al., 1999; Zhang et al., 2004).

Starch branching enzymes (SBE) cleave an existing α-1,4 linkage on the non-reducing end of an existing amylopectin chain, and re-attach the removed glucose subunit to the sixth carbon of another glucose subunit in an existing chain, forming a α-1,6 linked branch (Hannah, 2005). The coordination of the three known SBEs, SBEI, SBEIIa, and SBEIIb produces branches at regular intervals in amylopectin molecules (Mizuno et al., 2001; Rahman et al., 2001),

forming the basis for the characteristic structure of amylopectin. Only SBEIIb has been described in maize, which is encoded by the *Amylose extender1* (*ae1*) gene (Vineyard and Bear, 1952). In homozygous *ae1* mutants, the synthesis of amylopectin is impaired by reducing the formation of branches, resulting in a relative increase in amylose in the starch granule (Hedman and Boyer, 1982; Kim et al., 1998). The functions of SBEI and SBEIIa do not appear to compensate for the loss of SBEIIb (Mizuno et al., 2001; Rahman et al., 2001).

The final class of enzymes that is essential for normal starch production in cereals such as maize are the starch debranching enzymes (DBE). While the branching activity of BEs is necessary for the formation of amylopectin, DBEs are known to be important in refining the structure of the amylopectin molecule to allow it to crystallize (Burton et al., 2002; Delatte et al., 2005). There are two classes of DBE: isoamylase-types and pullulanase-types, which have similar but distinct functions (James and Myers, 2009). Both cleave α-1,6 linked glucose subunits from glucose polymers, however the activity of isolamylase-type DBEs is restricted to amylopectin, while pullulanase-type DBEs primarily act on pullulans. Their activity has not been fully described, but the most widely accepted model is the glucan-trimming model, which posits that DBEs remove specific branches to facilitate efficient packaging of the starch granule (Myers et al., 2000; Nakamura, 2002). In maize, ISA1 is encoded by the *Sugary1* (*Su1*) locus, and homozygous *su1* mutants have increased endosperm sugar levels, while producing an alternate form of starch called phytoglycogen or water-soluble polysaccharide (WSP) at the expense of amylopectin (Morris and Morris, 1939; James et al., 1995). The *su1* genotype is the basis for modern sweet corn, and will be described in greater detail in the a later section. A second isoamylase-type DBE isoform, *Isa2* has been described, along with the pullulanase, *Zpu1* (Beatty et al. 1999), and their interactions with *Su1* will also be discussed in that section.

The formation of a water-insoluble crystalline starch granule is complex and is not well understood (James and Myers, 2009). Amylose tends to form single and double-helices (French, 1984), while amylopectin is arranged in semi-crystalline structures. The organized, branched nature of amylopectin is responsible for its ability to densely pack glucose molecules in the starch granule (James and Myers, 2009; French, 1984). The branch clusters in adjacent amylopectin molecules align with each other, forming double-helices (James et al., 2003), which further arrange into lamella in concentric "growth rings" in the starch granule (Smith, 2001) that are hypothesized to grow outward from the center (Myers et al., 2000). Where the branch clusters align, crystalline lamella is formed, and where the gaps between clusters and the branch points are found, the lamella are considered amorphous. The level of packing of the short amylopectin chains determines what type of semi-crystalline structures are present, which can be types A, B, or C (Imberty et al., 1988; Imberty et al., 1991). Maize and other cereal starches are solely of the A-type, which is characterized by densely-packed hydrophobic granule with minimal bound water (James et al., 2003).

1.4 The origins of sweet corn and *sugary1 (su1)* in maize

Sweet corn is one of the most familiar and sought-after forms of maize, and was first described in written form in 1810 (Erwin, 1951). Unlike the more common form of maize, field corn, sweet corn is eaten at a stage when the developing kernels are still immature. The free sugars present in the endosperm of the kernels gives the maize its sweet taste, the degree of which can vary between varieties. While many traits differ between sweet and field corn such as plant growth habits and characteristics, and kernel tenderness, texture, and flavor, the most important difference is the presence of mutations in key starch metabolism genes that increase

the sweetness of the endosperm. The most important of such genes is the *Sugary1* locus, which encodes an isoamylase-type starch debranching enzyme (DBE) on chromosome 4. Recessive mutant alleles of this gene (*su1*) accumulate more sucrose in the developing endosperm as well as other changes that underlie the sugary sweet corn type.

Maize varieties containing *su1* alleles have been known to exist prior to the European colonization of the Americas, but there has been some debate regarding the particulars of its origin, and of sweet corn itself. There were two main competing theories regarding its origin, which were debated in the 1900s, which molecular evidence has helped answer considerably. The first was that it descended from a pre-Contact variety of maize and spread through crossing with other varieties of maize, and the second is that there were multiple origins of recessive sugary alleles.

Although *su1* varieties of maize existed in pre-Contact times, it was not widely grown, nor often eaten in the manner that modern sweet corn is eaten. The grain was usually ground into a flour for making confections or for use in making alcoholic beverages (Tracy 2001). Several candidate sources of the *su1* recessive allele were identified in the early 1900s, namely the Chullpi sweet corns of South America, and the Maize Dulce found in what is present-day Mexico. Several researchers agreed that it was likely that Maize Dulce was descended from the Chullpi complex, owing in part to the unique morphological characteristics of the ear such as high row number, and its high-altitude adaptations. These along with other uncommon characteristics of *su1* maize led Mangelsdorf and Galinat to argue that modern sweet corns descended from Maize Dulce (Mangelsdorf 1974, and Galinat 1971). Other evidence included the fact that *su1* mutations can lead to splitting and infection of the developing kernel in some

genetic backgrounds (Tracy, 1990b), suggesting that it would be difficult to identify and fix multiple different *su1* alleles independently.

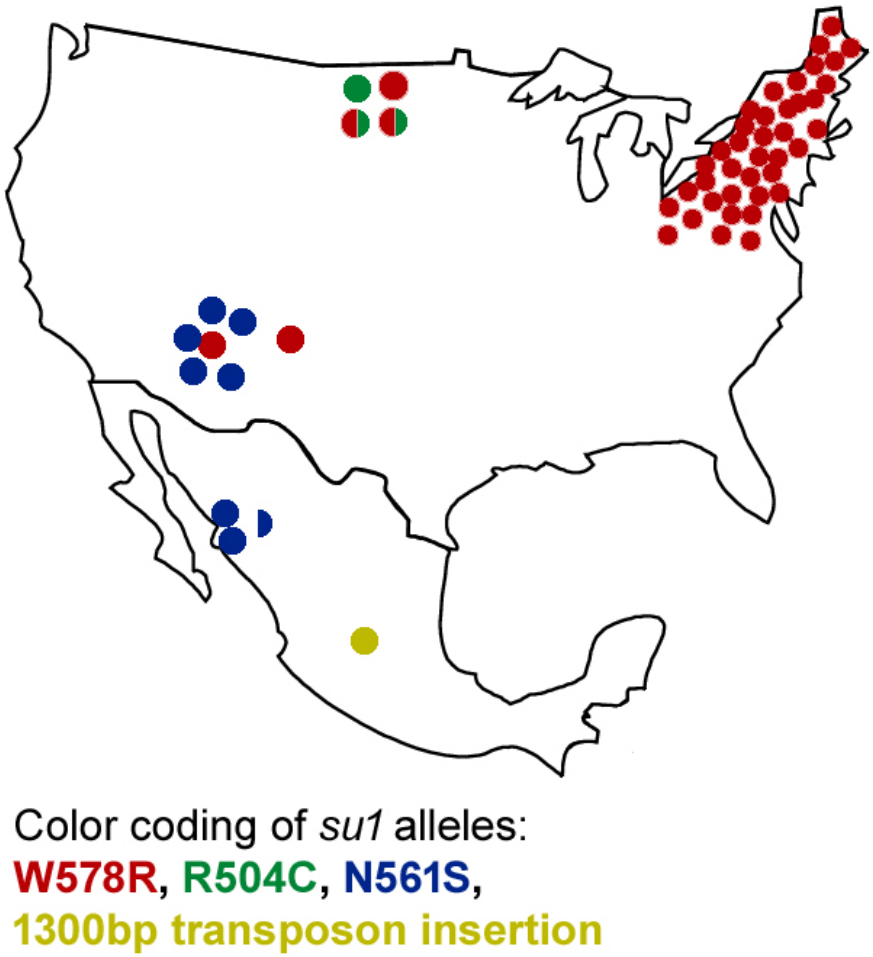
This theory, although it is parsimonious in these respects, requires accepting that the *su1* maize has been transported long distances, and become adapted to multiple different environments, which can be difficult if the genetic background is considered an important part of the argument for its origin. A variety of *su1* maize grown in Mexico that is adapted to lowlands, known as Dulcillo de Noroeste, was believed to be a cross between an arid, lowland-adapted popcorn and Maize Dulce (Wellhausen et al 1952).

The other theory on the origin of sugary endosperm maize was chiefly argued by Erwin (1951). Although he described a pre-Contact ear of sweet corn in North America (Erwin 1971), he was unconvinced that it was important in the origin of sugary maize in New England. He observed that spontaneous mutations for sugary endosperm have occurred in field corn varieties, and given the relative lack of records for sweet corn in New England (both written and in the form of preserved ears or seed), argued in favor of multiple independent origins for sugary alleles. In particular, sugary-type maize in North America was believed to be related to northern flint maize (Tracy 2001). This theory on multiple origins was parsimonious from the standpoint that if *su1* maize was present before the 1800s in New England, or traveled there at the time, that there should be some record of this. Also, this would explain the presence of the sugary phenotype in maize adapted to different environments (including day length), but did not explain the unique characteristics of Maize Dulce that were similar to Chullpi maize.

Due to the difficultly of determining if there were different alleles for the *sugary1* locus, and the problems associated with determining the origins of an allele at a specific locus from the presence or absence of other unlinked traits, this debate went largely unresolved until the

Sugary1 locus was mapped, and sequencing of different alleles was possible. Tracy et al. (2006) sequenced 57 accessions of sweet corn from North America (including the Northeast, Southwest and North Central regions), Mexico (Guanajuato, Sinaloa, and Sonora), and Peru. These cultivars included many modern sweet corn varieties as well as examples of the Chullpi, Maize Dulce, Dulcillo de Noroeste, and Hopi sweet corns from the Southwestern United States (Figure 1.2).

Figure 1.2



The geographic distribution of *su1* mutant alleles, coded by color. Mixed accessions are indicated by split circles. Exact geographic locations for the majority of the northeastern U.S. accessions are unknown. A fifth allele, from the Peruvian accessions, is not depicted in this figure. (Adapted from Tracy et al., 2006)

The results of this study indicated that there were five major recessive alleles that were distinct from each other in their location in the *Sugary1* locus. These alleles largely assorted according to geographic region, strongly arguing in favor of independent origins of *su1* alleles. The W578R allele was found in all of the Northeastern US cultivars as well as present somewhat in the Southwestern and North Central cultivars. A unique mutation, R504C, was also present in the North Central varieties. The N561W mutation was prevalent in the Southwestern cultivars and the Docillo de Noroeste varieties, while a transposon insertion mutation was present in the Maize Dulce and one Docillo de Noroeste cultivar. They explained this segregating variety as a later cross between two independently-derived cultivars. The fifth allele, which they were unable to identify through sequencing, was present in the two Peruvian Chullpi accessions tested (Tracy et al, 2006)

This was the first study to systematically survey *sugary1* alleles and their geographic distributions. Although additional accessions from Chullpi, Maize Dulce, and Docillo de Noroeste would strengthen the case against singular regional origins for some alleles for sugary, the case was made strongly for the independent origin theory. Due to the large number of sequenced accessions from North American cultivars of sugary maize, it is very certain that Erwin was correct that sugary maize has arisen multiple times in the history of maize cultivation.

1.5 Biology of *sugary1 (su1)* in maize

Although the *sugary1* locus was important in sweet corn breeding and mutations were easily identifiable, it was not until the gene was cloned in 1995 that its function could be confirmed (James et al, 1995). Prior research indicated that recessive *sugary1* mutants had a significant effect on the carbohydrate composition of maize endosperm, altering the ratios of

different starches and sugars. The primary effect of homozygous *su1* alleles is to accumulate sucrose and other simple sugars in the immature kernel, along with the production of the water-soluble polysaccharide (WSP) known as phytoglycogen. The increase in simple sugars along with phytoglycogen results in the phenotype characteristic of sugary maize, notably its sweetness and creamy texture. In addition to these changes, the content of amylopectin-type starch is reduced, and most research on the amylose starch fraction has indicated it is increased by *su1*, while some indicates a slight reduction. Overall, the total carbohydrate content of *su1* kernels is not significantly less than normal *Su1* kernels because most decreases in one carbohydrate are matched by increases in others. When dried, homozygous *su1* kernels are translucent and slightly wrinkled. This locus displays complete dominance, as kernels homozygous for *su1* are necessary to observe the phenotype (Tracy, 2001).

As the major difference in polysaccharides between *su1* and *Su1* endosperm is the replacement of amylopectin with phytoglycogen, it was hypothesized that the loss of SU1 function caused the conversion of amylopectin to phytoglycogen. Amylopectin is a highly-organized branched polysaccharide, composed of α-(1→4)-linked chains of glucose which are joined together by α-(1→6) branch linkages, as is phytoglycogen. However, the main difference in molecular structure between these two polysaccharides is with respect to the ratio of each type of linkage, which refers to the degree of branching (Ball et al, 1996). Phytoglycogen contains significantly more α-(1→6) linked branches than amylopectin, which means that it is more highly branched (James et al, 2003). This led to the hypothesis, now confirmed, that the *Sugary 1* locus coded for a starch debranching enzyme (Pan and Nelson, 1984), the loss of which results in an alteration of the ratio of starch branching and debranching enzymes during starch synthesis, leading to an increase in the number of branches in the resulting molecules.

James et al. (1995) used a *Mutator* (*Mu*) transposable element strategy to induce a new recessive mutation for *Sugary 1*, known as *su1-R4582::Mu1*, which identified sequence that cosegregated with *Sugary 1*. The sequence obtained from their investigation aligned to other known starch debranching enzymes in prokaryotes, most similarly to an isoamylase-type DBE that cleaves α-(1→6) linkages. They found that the gene was also expressed in the developing endosperm, and that the recessive *su1* reference allele was not caused by a large deletion. Further characterization of the *Sugary1* locus obtained a complete null and a mild allele for *su1* (Dinges et al. 2001).

Previously, starch DBEs were not believed to be involved in starch biosynthesis but only degradation. The function of *Sugary1* was further explored by Rahman et al. (1998), which found that the normal 79 kD SU1 enzyme hydrolyzes α-(1→6) linkages in branched polysaccharides. This confirmed that SU1 is an isoamylase-type DBE, which cleaves α-(1→6) linkages in glycogen, in contrast to the pullulanases which tend to cleave α-(1→6) linkages in pullulan and have little to no activity against glycogen. They also noted that the *su1* allele had not lost its isoamylase activity (Rahman et al, 1998). This agrees with other studies of *sugary1* homologs in other species such as rice (Kubo et al, 1999).

The discovery that the SU1 protein had isoamylase activity disconfirmed the prediction made by Pan and Nelson (1984) that it was a pullulanase-type DBE because they observed a loss in activity of this class of enzymes in *su1*lines. To resolve the disagreement between the apparent loss in pullulanase activity and the known identity of SU1, Rahman et al. (1998) proposed that the loss in *su1* function could either reduce the production of one or more pullulanase-type DBEs, or that the two DBEs associate in a complex of several enzyme subunits that include both

types of DBE. Mutations for *su1* might therefore reduce the activity of a pullulanase that is part of this complex.

The hypothesis that pullulanase production could be affected by *su1* was soon supported with the discovery of the corresponding pullulanase in maize endosperm, *Zpu1* (Beatty et al. 1999). In the presence of *su1*, the transcript for *Zpu1* is present in normal levels, but the quantity of the active form of ZPU1 protein is substantially reduced, indicating a post-transcriptional pleiotropic effect. Beatty et al. suggest that enzymatic modification of ZPU1 by the SU1 protein may be another likely explanation and note that the two enzymes do not associate in the same chromatographic fractions, suggesting they may not associate with each other in a complex.

Following the discovery that isoamylases have been found in complexes, Kubo et al (2010) generated a new mutant for *su1* named *su1-P*, and compared it to a mutant for *isa2*, another isoamylase (ISA2). They determined that ISA1 exists as a homomeric complex as well as a heteromeric complex with ISA2. Thus, the gene products of *Su1* and *Isa2* can interact as part of a multisubunit complex. In addition, Tracy et al. (2006) noted that the three amino acid substitution mutants they identified for *su1*, when mapped to a model of a prokaryotic isoamylase, fell on highly-conserved residues that were in one cleft outside the active site of the enzyme (Figure 1.2). This could potentially be a site that is involved in binding to other complex subunits. While neither of these findings confirm the isoamylase-pullulanase complex hypothesis, they do show that the SU1 protein can form enzymatic complexes, and that the activity of such complexes may be necessary for coordinated debranching activity that results in normal starch biosynthesis in maize endosperm.

The two major effects of the *su1* recessive allele of increased sucrose content and conversion of amylopectin to phytoglycogen, like many other metabolic traits in plants, can be

modified by other genes in varying genetic backgrounds. While the sucrose content of *su1* lines is not especially high, when combined with some other recessive alleles the sucrose content can be further increased. The *shrunken2* allele (*sh2*) increases sucrose content significantly, similar to *sh2* by itself, however the levels of phytoglycogen characteristic of *su1* are significantly reduced in this combination. Recessive alleles of *amylose extender1* (*ae1*) and *brittle2* (*bt2*) also perturb the production of phytoglycogen when combined with *su1*. In contrast, *waxy1* (*wx1*), *dull1* (*du1*), and *brittle1* (*bt1*) maintain the levels of phytoglycogen when combined with *su1* (Andrew et al., 1944, Cameron and Cole, 1959, and Hannah and Basset, 1977). Another important modifier of *sugary1* is *sugary enhancer1*, which will be discussed in the final section.

Selection for pseudo-starchy endosperm in a *su1* population caused dramatic changes in carbohydrate proportions (Tracy and Chang, 2007). After six cycles of selection, sucrose was reduced by 83%, glucose by 93%, and phytoglycogen by 50%, while total starch was increased by 17%. When crossed into seven inbred lines of field corn, *su1* inbreds had anywhere from 13% to 26% total sugars, indicating that there were significant modifiers for this aspect of the phenotype. The ability to breed for altered starch content and germination in *su1* sweet corns suggests that further uncharacterized modifiers of the sugary phenotype may exist.

1.6 Previous research on *sugary enhancer1 (se1)*

In the 1970s, A.M. Rhodes discovered a new recessive modifier of *sugary1* called *sugary enhancer1 (se1)*, which had a distinctive phenotype valued in sweet corn (Gonzales et al. 1974, Ferguson et al. 1978). When present in the same line as *su1*, *se1* doubles the sugar content of the kernel at the sweet corn eating stage, while maintaining levels of WSP and possessing a creamy texture. These traits, along with a tender pericarp and lighter kernel color, and good flavor

became known as “sugary enhanced” (or simply *se*) sweet corn, and this combination of traits has been bred into many varieties of sweet corn, particularly for fresh market sweet corn that may be found in farmer’s markets and roadside stands (Gonzales et al. 1976). In accordance with maize gene nomenclature, the gene name is formally known as *Sugary enhancer1*, or *Se1*.

The *se1* trait was discovered in a line called IL677a, which was developed from a three-way cross between two sweet corn lines and a Bolivian Corioco flour corn population, Bolivia 1035. *IL677a: (Bolivia 1035 x IL44b) x IL442a*

The Corioco flour corn Bolivia 1035 was reported by Schultz & Juvik (2004), to be the probable source of the *se1* allele. As Bolivia 1035 is a population, it may possess diverse modifiers of carbohydrate metabolism in the endosperm. Also worthy of note is IL44b, which derives from an uncertain cross between two Narrow Grain Evergreen sweet corns or between a Narrow Grain Evergreen and Country Gentlemen variety (Gerdes et al. 1993). Narrow Grain Evergreen lines, derived from Stowell’s Evergreen, were so named because they maintained their eating quality longer than other varieties, and had a slower dry-down (Tracy, 1990a).

Several studies have examined the impact of *se1* on kernel carbohydrates at the eating stage (18-22 DAP) and in dry seeds. In addition to higher sugar levels and the production of WSP at the expense of amylopectin, elevated levels of maltose were also observed (Fergusen et al., 1979). However, Azanza et al. (1996) instead observed reduced levels of phytoglycogen with *se1*, and elevated maltose concentrations were not observed in all *se1* genotypes (La Bonte and Juvik, 1990; Carey et al., 1982), so these traits may be due to closely linked genes and not *se1* itself. The increase in sugar content, high levels of WSP, low amylopectin content, and longer dry-down are the most consistent traits associated with *se1* (Schultz and Juvik, 2004).

The first study that genetically mapped *se1* was conducted by Le Bonte and Juvik (1991), and used B-A translocation stocks to place it on the long arm of chromosome 4. However, a subsequent study by Tadmor et al. (1995) using restriction fragment length polymorphisms (RFLP) placed *se1* on the distal end of the long arm of chromosome 2. This location is the most consistent result and has been confirmed by other studies (Juvik et al., 2003). Schultz and Juvik (2004) speculate that some of the other loci on chromosomes 3 and 6 as identified in Tadmor et al. (1995) may be modifiers that are also important for sugary enhanced varieties of sweet corn.

There are some questions about *sugary enhancer1* that remain unresolved.

1. Where is *se1* located in the maize genome?
2. Does *se1* align to previously-described starch metabolism genes in other species?
3. What is the role of *se1* in starch metabolism?
4. What is the origin of the *se1* allele?
5. Can a reliable codominant marker be developed to assist with breeding *se1* into new sweet corn varieties?

This research was undertaken to resolve these questions. It will be important to precisely map the causative locus in the maize genome and develop a marker that can be used to facilitate breeding. This would be of prime importance for both basic research on the function of this gene and on the details of carbohydrate metabolism in maize, but could also be used for marker-assisted selection in sweet corn breeding programs to develop improved sweet corn varieties. This information will also enable research into the origins of *se1* and how it became a part of the history of sweet corn. While many of the basic details of starch metabolism in maize and other crops have been determined, there is still much that is unknown and due to its unique characteristics *sugary enhancer1* may provide new insights into this process.

1.7 REFERENCES

Andrew RH, Brink RA, Neal NP. 1994. Some effects of waxy and sugary genes on endosperm development in maize, *J. Agric. Res.,* 69, 355.

Azanza F, Klein BP, and Juvik JA. 1996. Sensory characterization of sweet corn lines differing in physical and chemical composition. *Food Sci.*, 1996 61: 253-257.

Bae, J. M., M. Giroux, and L. C. Hannah. 1990. Cloning and characterization of the *Brittle-2* gene of maize. *Maydica*. 35:317-322.

Beatty MK, Rahman A, Cao H, Woodman W, Lee M, Myers AM, James MG. 1999. Purification and molecular genetic characterization of ZPU1, a pullulanase-type starch-debranching enzyme from maize. *Plant Physiol*. 119(1):255-66.

Berger, F. 1999. Endosperm Development. *Curr. Opinion Plant. Biol*. 2: 28-32.

Bhave, M. R., S. Lawrence, C. Barton, and L. C. Hannah. 1990. Identification and molecular characterization of Shrunken-2 cDNA clones of maize. *Plant Cell*. 2:581-588.

Bosnes, M., F. Weideman, and O. A. Olsen. 1992. Endosperm differentiation in barley wildtype and sex mutant. *Plant Journal*. 2: 661-674.

Burton, R.A., H. Jenner, L. Carrangis, B. Fahy, G.B. Fincher, C. Hylton, D.A. Laurie, M. Parker, D. Waite, and S. Van Wegen. 2002. Starch granule initiation and growth are altered in barley mutants that lack isoamylase activity. *Plant Journal*. 31:97-l 12.

Cameron JW and Cole DA. 1959. Effects of the genes su, sh2, and du on carbohydrates in developing maize kernels, *Agron J*., 51, 424.

Cameron, J.W. and H.J. Teas. 1954. Carbohydrate relationships in developing and mature endosperms of brittle and related maize genotypes. *Am. Journ. of Bot*. 41: 50-55.

Cao, H., T.D. Sullivan, C.D. Boyer, and J.C. Shannon. 1995. Bt1, a structural gene for the major 39-44 kDa amyloplast membrane polypeptides. *Physiologia Plantarum*. 95: 176-186.

Cao, H., M.G. James, and A.M. Myers. 2000. Purification and characterization of soluble starch synthases from maize endosperm. *Arch. Biochem. Biophys*. 373:135-146.

Carey EE, Dickinson DB, and Rhodes AM. 1984. Sugar characteristics of sweet corn populations from a sugary enhancer breeding program. *Euphytica* 33 609-622.

Carey EE, Rhodes AM, and Dickinson DB. 1982. Post-harvest levels of sugars and sorbitol in sugary enhancer (*su se*) and sugary (*su Se*) maize. *HortScience* 17:241-242

Cheng, W. H., E. W. Taliercio, and P. S. Chourey. 1996. The *Miniature1* seed locus of maize encodes a cell wall invertase required for normal development of endosperm and maternal cells in the pedicel. *Plant Cell*. 8:971-983.

Chourey, P. S., and O. S. Nelson. 1976. The enzymatic deficiency conditioned by the shrunken-1 mutations in maize. *Biochemical Genetics*. 14: 1042-1055.

Chourey, P. S., and E. W. Taliercio. 1994. Epistatic interaction and functional compensation between the two tissue- and cell-specific sucrose synthase genes in maize. *PNAS.* 91: 7917-7921.

Deatherage, W. L., M. M. MacMasters, and C. E. Rist. 1955. A partial survey of amylose content in starch from domestic and foreign varieties of corn, wheat, and sorghum and from other starch bearing plants. *Trans. Am. Assoc. Cereal Chem*. 13:31-42.

Denyer, K., P. Johnson, S. C. Zeeman, and A. M. Smith. J. 2001. The control of amylose synthesis. *Plant Physiol.* 158:479-487.

Dinges, J.R., C. Colleoni, A.M. Myers, and M.G. James. 2001. Molecular structure of three mutations at the maize sugary1 locus and their allele-specific phenotypic effects. *Plant Physiol.* 125:1406–1418.

Erwin, AT. 1934. A rare specimen of Zea mays var. saccharata, *Science,* 79: 589.

Erwin, AT. 1951. Sweet corn – Mutant or historic species? Econ. Bot. 5: 302.

Esau, K. 1997. *Anatomy of Seed Plants*. Wiley and Son, Inc. New York.

Ferguson JE, Dickinson DB, and Rhodes AM. 1979. Analysis of endosperm sugars in a sweet corn inbred (Illinois 677a) which contains the sugary enhancer (*se*) gene and comparison of *se* with other corn genotypes. *Plant Physiol.* 63(3):416-20.

Ferguson JE, Rhodes AM, and Dickinson DB. 1978. The genetics of sugary enhancer (*se*), an independent modifier of sweet corn (*su*). *J Hered* 69 (6): 377-380.

French, D. 1984. Organization of starch granules, p. 183-247. *In* R.L. Whistler, J.N. BeMiller, and E.F. Paschall (eds.) Starch: Chemistry and technology. Academic Press, Orlando, FL, USA.

Friedman, W.E. 1998. The evolution of double fertilization and endosperm: An "historical" perspective. *Sex Plant Reprod* 11:6-16.

Friedman, W. E. 2001. Development and evolutionary hypotheses for the origin of double fertilization and endosperm. *Comptes Rendus de l Academie des Sciences, Sciences de la Vie (Paris)*. 324:59-567

Galinat WC. 1971. The evolution of sweet corn, *Mass. Agric Exp. Sta. Bull*., 591.

Gerdes JT, Behr CF, Coors JG, and Tracy WF. 1993. Compilation of North American Maize Breeding Germplasm. *Crop Science Society of America.*

Gonzales JW, Rhodes AM, and Dickinson DB. 1974. A new inbred with higher sugar content in sweet corn. *Hortscience* 9(1).

Gonzales JW, Rhodes AM, and Dickinson DB. 1976. Carbohydrate and enzymatic characterization of a high sucrose sugary inbred line of sweet corn. *Plant Physiol.* 58:28-32

Guignard L. 1899. Sur les antherozoi'des et la double copulation sexuelle chez les vegetaux angiospermes. *C R Acad. Sci. Paris* 128:864-871.

Hannah, L. C. 1997. Starch synthesis in the maize endosperm. p. 375-405. In B. A. Larkins and I. K. Vasil (eds.) *Advances in cellular and molecular biology of biology*, Vol. 4. Kluwer Academic Publisher. Dordrecht, The Netherlands.

Hannah LC and Basset MJ. 1977. Use of brittle-a gene in sweet corn breeding, *HortScience*, 12, 313.

Hannah LC. 2005. Starch synthesis in the maize endosperm. *Maydica.* 50: 497-506.

Hedman, K.D. and C.D. Boyer. 1982. Gene dosage at the amylose-extender locus of maize: effects on the levels of starch branching enzymes. *Biochem. Genet*. 20: 483-492.

Imberty, A., A. Buleon, V. Tran, and S. Peerez. 1991. Recent advances in knowledge of starch structure. *Starch/Starke* 43:375-384.

Imberty, A., H. Chanzy, S. Perez, A. Buleon, and V. Tran. 1988. The double-helical nature of the crystalline part of A-starch. *J. Mol. Biol.* 201:365-378.

James, M., D. Robertson, and A. Myers. 1995. Characterization of the maize gene *sugary1*, a determinant of starch composition in kernels. *The Plant Cell*. 7: 417-429.

James MG, Denyer K, and Myers AM. 2003. Starch synthesis in the cereal endosperm. *Current Opinion in Plant Biology.* 6:215–222.

James, M. G., and A. M. Myers. 2009. Seed starch synthesis. p. 439-456. In J.L. Bennetzen, and S.C. Hake (eds.) *Handbook of maize: Its biology*. Springer Science+Business Media, LLC. New York, NY.

Jeon J, Ryoo N, Hahn T, Walia H, and Nakamura Y. 2010. Starch biosynthesis in cereal endosperm. *Plant Physiology and Biochemistry* 48:383-392

Juvik, JA, Yousef GG, Han T, Tadmor Y, Azanza F, Tracy WF, Barzur A, and Rocheford TR. 2003. QTL influencing kernel chemical composition and seedling stand establishment in sweet corn with the *shrunken2* and *sugary enhancer*1 endosperm mutations. *J. Amer. Soc. Hort. Sci.* 128:864–875.

Katsuya, Y., Y. Mezaki, M. Kubota, and Y. Matsuura. 1998. Three dimensional structure of Pseudomonas isoamylase at 2.2 Å resolution. *J. Mol. Biol.* 281:885–897.

Kim, K., D. K. Fisher, M. Gao, and M. J. Guilitinan. 1998. Molecular cloning and characterization of the Amylose-Extender gene encoding starch branching enzyme IIB in maize. *Plant Mol. Biol*. 38:945-956.

Kiesselbach, T.A. 1949. The structure and reproduction of corn. *Nebr. Agr. Exp. Sta. Res. Bul* 161:3-93.

Kubo A, Fujita N, Harada K, Matsuda T, Satoh H, Nakamura Y. 1999. The starch-debranching enzymes isoamylase and pullulanase are both involved in amylopectin biosynthesis in rice endosperm. *Plant Physiol* 121:399–409

Kubo A, Colleoni C, Dinges JR, Lin Q, Lappe RR, Rivenbark JG, Meyer AJ, Ball SG, James MG, Hennen-Bierwagen TA, Myers AM. 2010. Functions of Heteromeric and Homomeric Isoamylase-Type Starch-Debranching Enzymes in Developing Maize Endosperm. *Plant Physiology* vol. 153 no. 3 956-969

La Bonte DR, Juvik JA. 1990. Characterization of *sugary1 (su1) sugary enhancer (se)* kernels in segregating sweet corn populations. *J. Am. Soc. Hort. Sci.* 155(1) 153-157.

La Bonte DR, Juvik JA. 1991. *Sugary enhancer (se)* gene located on the long arm of chromosome 4 in maize (Zea mays L.), *Hereditas* 82:176-178

Laughnan JR. 1953. The effect of the sh2 factor on carbohydrate reserves in the mature endosperm of maize. *Genetics*. 38: 485-499.

Lopes, M.A., and B.A. Larkins. 1993. Endosperm origin, development, and function. *Plant Cell* 5:1383-1399.

Mangelsdorf PC. 1974. *Corn; Its Origin, Evolution, and Improvement*, Belknap/Harvard University Press, Cambridge MA

Mitsui, T., K. Itoh, H. Hori, and H. Ito. 2010. Biosynthesis and degradation of starch. *Bull. Facul. Agric. Niigata Univ*. 62: 49-73.

Mizuno, K., E. Kobayashi, M. Tachibana, T. Kawasaki, T. Fujimura, K. Funane, M. Kobayashi, and T. Baba. 2001. Characterization of an isoform of rice starch branching enzyme, RBE4, in developing seeds. *Plant Cell Physiol* 42:349-357.

Morris, D. Z., and C. T. Morris. 1939. Glycogen in the seed of *Zea mays*. *Journal of Biological Chemistry.* 130: 535-544.

Myers, A. M., M. K. Morell, M. G. James, and S. G. Ball. 2000. Recent progress toward understanding the amylopectin crystal. *Plant Physiology*. 122: 989-997

Nakamura, Y. 2002. Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: Rice endosperm as a model tissue. *Plant Cell Physiol* 43:718-725.

Nelson, O. E., and D. Pan. 1995. Starch synthesis in maize endosperms. *Annual Review of Plant Physiology and Plant Molecular Biology*. 46: 475-496.

Pan D, and Nelson OE. 1984. A debranching enzyme deficiency in endosperms of the sugary-1 mutants of maize. *Plant Physiol* 74: 324–328

Preiss, J. 1991. Biology and molecular biology of starch synthesis and its regulation. *Oxford Surveys of Plant Molecular and Cell Biology*. 7: 59-114.

Rahman A, Wong K, Jane J, Myers AM, James, MG. 1998. Characterization of SU1 Isoamylase, a Determinant of Storage Starch Structure in Maize. Plant Physiology, Vol. 117, No. 2 425-435

Rahman, S., A. Regina, Z. Li, Y. Mukai, M. Yamamoto, B. Kosar-Hashemi, S. Abrahams, and M.K. Morell. 2001. Comparison of starch-branching enzyme genes reveals evolutionary relationships among isoforms. *Characterization of a gene for starch-branching enzyme Ila from the wheat genome donor* Aegilops tauschii. Plant Physiol. 125:1314-1324.

Raven PH, Evert RF, and Eichorn S. 1992. *Biology of Plants*. Worth Publishers. New York.

Sabelli PA, Nguyen H, Larkins BA. 2007. Cell Cycle and endosperm development. In *Dirk Inze (Ed.) Cell Cycle Control and Plant Development*. Blackwell Publishing Ltd.

Schultz JA, and Juvik JA. 2004. Current models for starch synthesis and the sugary enhancer 1 mutation in Zea mays. *Plant Physiology & Biochemistry*. 42(6): 457-464.

Schweizer L, Yerk-Davis GL, Phillips RL, Srienc F, and Jones RJ. 1995. Dynamics of maize endosperm development and DNA endoreduplication. *Proc. Natl. Aced. Sci*. 92: 7070-7074.

Smith AM. 2001. The biosynthesis of starch granules. *Biomacromolecules*. 2:335-341.

Spielbauer G, Margl L, Hannah C, Römisch W, Ettenhuber C, Bacher A, Gierl A, Eisenreich W, and Geschel U. 2006. Robustness of central carbohydrate metabolism in developing maize kernels. *Phytochemistry*. 67: 1460-1475.

Sprague GF. 1932. The nature and extent of hetero-fertilization in maize. *Genetics* 17: 358–368.

Sprague, GF, and B. Brimhall. 1943. Some effects of the waxy gene in corn on properties of the endosperm starch. *J. Am. Soc. Agron*. 35:817-822.

Strasburger, E. 1879. Die angiospermen und die gymnospermen. *Jena.*

Strasburger, E. 1900. Einige bemerkungen zur frage nach der "doppelten befruchtung" bei den angiospermen. *Bot. Zeit* 58:293-316.

Sullivan, T.D., L.I. Strelow, C.A. Illingworth, R.L. Phillips, and O.E. Nelson. 1991. Analysis of maize *Brittle-1* alleles and a defective *Suppressor-Mutator*-induced mutable allele. Plant Cell. 3: 1337-1348.

Sullivan, T.D. and Y. Kaneko. 1995. The maize *brittle1* gene encodes amyloplast membrane polypeptides. *Planta*. 196: 477-484.

Tadmor Y, Azana F, Han T, Rocheford TR and Juvik JA. 1995. RFLP mapping of the *sugary enhancer1* gene in maize. *Theoretical Applied Genetics* 91, 489-494.

Teas, H.J. and A.N Teas. 1953. Heritable characters in maize: description and linkage of brittle endosperm-2. *Journal of Heredity*. 44: 156-158.

Tracy WF. 1990a. Breeding for rust resistance in sweet corn. *Midwest Food Processors Association Processing crops manual and proceedings*. Dep. of Horticulture, Univ. of Wisconsin, Madison. p. 25-29

Tracy W.F. 1990b Potential of field corn germplasm for the improvement of sweet corn. *Crop Sci.* 30, 1041—1045.

Tracy, W. F., 2001: Sweet corn. In: A. R. Hallauer (ed.), *Specialty Corns*, 2nd edn, 155—197. CRC Press LLC, Boca Raton, FL.

Tracy, W. F., and Y. M. Chang, 2007: Effects of divergent selection for endosperm appearance in a Sugary1 maize population. *Maydica*, 52, 71—79.

Tracy WF, Whitt SR, Buckler ES. 2006. Recurrent mutation and genome evolution: example of Sugary1 and the origin of sweet maize. *Crop Sci* 46(S1).

True, R.H. 1893. On the development of the caryopsis. *Botanical Gazette*. 18: 212-226.

Vilhar, B., A. Kladnik, A. Blejec, P. S. Chourey, and M. Dermastia. 2002. Cytometrical evidence that the loss of seed weight in *miniature1* seed mutant of maize is associated with reduced mitotic activity in the developing endosperm. *Plant Physiology*. 129: 23-30.

Vineyard, M. and R. Bear. 1952. Amylose content. *Maize Genetics Cooperation Newsletter*. 26:5.

Wellhausen EJ, Roberts LM, Hernandez, XE. 1952. *Races of Maize in Mexico*, Bussey Inst., Harvard Univ., Cambridge MA

Young, T.E., and D.R. Gallic 2000. Regulation of programmed cell death in maize endosperm by abscisic acid. *Plant Mol. Biol*. 42:397-414.

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Manuscript Title: Maize sugary enhancer1 (*se1*) is a presence-absence variant of a previously uncharacterized gene.

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Author contributions: CNH contributed to some of the early genetic mapping data and marker design. BDV performed the kernel and dry seed carbohydrate measurements. WFT generated the seed stocks used in mapping, and contributed to the analysis of *se1* origins.

CHAPTER 2: Maize *sugary enhancer1* (*se1*) is a presence-absence variant of a previously uncharacterized gene.

2.1 ABSTRACT

Starch metabolism and catabolism are important in diurnal cycling of carbohydrates in plant leaves, in gravitropic response, and in accumulation of starch in organs to support processes including plant germination, vegetative growth, and pollen germination. Central genes and proteins involved in starch metabolism have been characterized, but components of the pathway remain to be discovered. The recessive starch modifier *sugary enhancer1* (*se1*) has been utilized commercially in combination with *sugary1* (*su1*) to develop sweet corn cultivars with improved flavor, but has previously not been mapped to a single locus. The goal of this study was to characterize the *Se1* gene by map-based cloning of a naturally occurring allele. The sugary enhancer trait was Mendelized in a pair of near-isogenic lines developed by self-pollination in a genetic background homozygous for *su1*. The homozygous *se1* isoline is lower in starch, and higher in phytoglycogen and sugars than the homozygous *Se1* isoline. The *se1* allele is comprised of a 637 bp deletion that encompasses all of annotated gene model AC217415.3_FG004. This gene is specific to monocots, and the predicted protein does not include any characterized functional domains. The gene is expressed in B73 in the endosperm, embryo, and developing leaf. This discovery provides evidence of a previously uncharacterized mechanism that may lead to a deeper understanding of how different species of plants modulate starch metabolism.

2.2 INTRODUCTION

Starch is the most important storage carbohydrate in plants, and is a dominant source of energy for humans and livestock. Understanding the underlying genetics of starch metabolism is important for improving crops such as cereals and starchy vegetables. In the leaves, starch accumulates during the day as a result of photosynthetic activity, and is consumed during the night to supply tissues with energy. Carbohydrates are transported throughout the plant in the form of sucrose, which is converted into starch in the plastids of storage tissues that act as sinks, such as tubers, storage roots and leaves, and stems, to be later used as a source of energy for the plant or its progeny. In cereal crops, starch stored in the endosperm is the main energy source for the germinating seedling until it is able to produce energy through photosynthesis.

Starch consists of two main types, amylose, which consists of homopolymers of α-1,4 linked glucose subunits, and amylopectin, which adds α-1,6-linked branches to the α-1,4 linked chains, forming a highly organized, branched crystalline molecule. Amylopectin makes up the majority of starch in plant cells, and its production and degradation is a dynamic process conducted by a suite of enzymes that have been identified through studying mutant forms of these genes. These include starch synthases (SS), branching and debranching enzymes (BE, DBE), enzymes that prepare glucose subunits for incorporation into the growing molecule, such as ADP Glucose Phosphorylase (AGPase), and various regulatory proteins have been identified (Jeon et al. 2010).

The *Sugary1* (*Su1*) locus in maize encodes an isoamylase-type starch DBE which removes excess branches from growing amylopectin molecules, allowing the formation of an densely-packed starch granule (James et al., 2003). Loss of the debranching activity performed by the SU1 enzyme, in *su1* kernels increases the number of branches in the growing starch

molecules, leading to the production of the water-soluble polysaccharide (WSP) at the expense of amylopectin. WSP in maize endosperm produces a desirable creamy texture in sweet corn at the milk stage when sweet corn is harvested and eaten (Schultz & Juvik 2004, Tracy, 2006).

An important modifier of the *su1* phenotype, *sugary enhancer1* (*se1*), was discovered in the 1970s by A.M. Rhodes (Gonzales et al. 1974, Ferguson et al. 1978) and has been incorporated into many popular sweet corn varieties used in the fresh market. In many *su1* lines studied, *se1* approximately doubles the sugar content of the endosperm while maintaining levels of WSP and its creamy texture. "*se* type" sweet corn also has tender kernels, lighter color, and good flavor (Gonzales et al. 1976).

Previous studies have mapped *se1* to multiple loci in the maize genome, but the most consistent locus identified is on the distal end of the long arm of chromosome 2 (Tadmor et al. 1995; Juvik et al., 2003). Characterization of *se1* has been limited by complex genetic background effects on phenotypic expression. Consequently, in order to observe the presence or absence of *se1* for mapping purposes, its trait must be "Mendelized," which is to develop a stock that shows a clear phenotypic difference that follows a simple Mendelian pattern of inheritance (Davis et al, 2005).The goal of this study was to characterize *se1* using a map-based cloning approach.

2.3 MATERIALS AND METHODS

Genetic Stocks

Near-isogenic lines homozygous for *su1* but segregating for *se1* were developed from an initial cross between Wh8419 and Terminator, a sweet corn line heterozygous for *se1*. The F_1 progeny were self-pollinated, and the resulting F_2 were self-pollinated for eight additional

generations, selecting for segregation of a wrinkled kernel phenotype. F_2 seeds derived from self –pollination of the F_1 cross of the isolines segregate 3:1 smooth:wrinkled kernels consistent with the hypothesis that the sugary enhancer trait was Mendelized and segregates as a single, fully recessive gene (Figure 2.1). These materials, which gave rise to the mapping population used in this study, were maintained as heterozygotes and selected for low sugar content by single-seed descent.

Carbohydrate Analysis

To analyze kernel and leaf carbohydrates, homozygous *Se1* and *se1* plants from the mapping population were grown in the summer of 2011 in side-by side plots, planted 15 plants per 6 m row, with the rows spaced 76 cm apart. *Se1* and *se1* ears were self-pollinated on 7/10 and 7/14. Three random ears were harvested and frozen at -20° C for each genotype at 2-day intervals between 14 and 24 days after pollination (DAP). Whole seeds from mature ears (45 DAP) of *Se1* and *se1* genotypes that were grown side-by-side in 2006 and 2007 were also analyzed with three biological replications each. The kernels were freeze-dried and ground using a Udy mill with a 0.5 mm screen. The ground seed tissue was used to determine individual sugars and polysaccharides.

Sucrose, D-fructose, and D-glucose were measured using the Megazyme Sucrose, D-Fructose, and D-Glucose assay kit (catalog number K-SUFRG, Megazyme International Ireland, Ltd., Bray, Ireland). 100 mg of ground seed sample was placed in a 15 mL glass tube, which was suspended in water and centrifuged. The supernatant was removed and used for sugar analysis, which was conducted according to the manufacturer's directions and converted to mg g^{-1} of dry weight. Sucrose, D-fructose, D-glucose, and blank standards were included as controls.

Total polysaccharides and starch were measured using the Megazyme K-TSTA assay kit (catalog number K-TSTA, Megazyme International Ireland Ltd., Bray, Ireland). For both assays, 100 mg of ground seed sample was placed in a 15 mL glass tube, and were processed according to the manufacturer's directions and converted to mg g^{-1} of dry weight. Maize starch was included as a control for the enzymatic step while a glucose control and water sample were included for the glucose measurement. WSP was determined by subtracting the starch from the total mass of polysaccharide in the samples. The *Se1* and *se1* carbohydrate levels were compared using a 1-tailed student's T-Test, and the data from the 2006 and 2007 mature seeds was pooled.

Leaf tissue samples were taken from the leaf below the flag leaf, which were 10 inches in length, starting at 2 inches from the base of the leaf. Leaves were photographed and the samples were chopped into 1-inch lengths, flash-frozen, and transferred to -80° C. Three different plants were sampled for each genotype at each sample time point. Leaf samples were collected for a 24-hour time course every 3 hours, beginning at dawn (05:30) on July 8, and ending at dawn on July 9. Samples were also collected at 11:30 on several subsequent dates (July 11, 13, 14, 15, 17, 18 19). Leaf samples for carbohydrate analysis were chopped and lyophilized, and ground into powder to obtain 100 mg of dry tissue. Leaf carbohydrates were analyzed according to the protocol outlined in Sekhon et al. (2012) at the GLBRC facility at Michigan State University. Levels of carbohydrates in the leaves of *Se1* and *se1* plants were compared using a student's T-test and applying a Bonferroni correction.

Genetic Analysis

DNA oligonucleotide primers were designed using genomic DNA from the B73 reference sequence obtained from maizesequence.org. Genes and gene fragments were identified

based on evidence of expression from Expressed Sequence Tags (ESTs), and/or by gene models in open reading frames predicted by FGENESH. Primers were designed using Primer3 to flank introns. Homozygous smooth (*Se1/Se1*) and homozygous wrinkled (*se1/se1*) seeds from the mapping population were germinated from three different ears to obtain technical replications for each genotype, and DNA was extracted with a modified CTAB protocol (Doyle and Doyle, 1990). Primer pairs were amplified by Polymerase Chain Reaction (PCR) with a touch-down protocol using HotStarTaq. High GC-content sequences were amplified using a touch-down protocol using AccuPrime. Successful amplifications were Sanger sequenced, and the data was analyzed using BioEdit. Polymorphisms were screened as markers against the mapping population of *se1/se1* seeds using direct sequencing, RFLP, and KASPar SNP assays (Table 2.3).

Expression Analysis

RNA was extracted from B73 plants at specific stages of development using a Trizol extraction protocol. cDNA was synthesized using the SuperScript III kit according to the manufacturer's directions. GRMZM2G129817 was selected as a low-expression control from Sekhon et al. (2011), with an average expression of 67 FPKM across all tissues (range: 32-120 FPKM), and primers were designed flanking a small intron to produce a small (137bp) band from cDNA, and produce a larger (~250 bp) band that includes the intron from genomic DNA. The semi-quantitative PCR reaction was performed using AccuPrime with 20 cycles of touchdown followed by 22 cycles with a constant annealing temperature, which were determined through testing to be the optimal number of cycles to reveal differences in expression.

Bioinformatic Analysis

Candidate genes in the region were BLASTed for alignments against Maize GDB, TIGR, Plant GDB, Gramene, and GenBank. Searches were performed on 11-10-2010, and repeated on 2-15-2013 and 1-09-2014. DNA and protein sequences were analyzed using BioEdit. Protein motif and localization analysis was performed using Predict Protein (www.predictprotein.org), Motif Scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan), TargetP (www.cbs.dtu.dk/services/TargetP/) and ChloroP (www.cbs.dtu.dk/services/ChloroP/) on 10/11/2011, and PSORT II on 1/09/2012, and repeated on 04/02/2014. Synteny was investigated using SyMAP Synteny Browser on 04/04/2014 (www.symap.org).

2.4 RESULTS

Carbohydrate analysis

Mature seeds of the homozygous *se1/se1* isoline contained 79% more water-soluble polysaccharides, approximately 53% the starch, and 29% more total sugars than the *Se1/Se1* counterpart (Figure 2.2, Table 2.1) consistent with the expected effect of the *se1* modifier ($p < 0.01$). A similar effect was observed at 22 DAP, however, the differences were not as significant. Total sugars were not significantly different at 22 DAP ($p = 0.12$), but WSP was greater ($p = 0.0104$) and Starch was significantly lower ($p << 0.001$).

Since we detected expression of *Se1* in leaves, we sought to determine if there was an effect of *se1* on leaf starch and free sugar metabolism throughout a 24-hour time course (Figure 2.5). A diurnal pattern of sucrose, free sugar, and starch accumulation was observed beginning at dawn, with a decline in leaf carbohydrates beginning at dusk. Overall, no clear differences were observed between the *Se1* and *se1* isolines. Although *se1* had higher starch levels (3.2% vs 2.5%

by dry weight) at timepoint 14:30, this was not significant after applying a Bonferroni correction for multiple testing to a two-tailed T-test ($p = 0.044 > 0.0055$ after Bonferroni correction).

Genetic mapping

A total of 121 SSR markers throughout the maize genome (Table 2.2) were used to compare the *Se1* and *se1* homozygous isolines. A single marker, UMC 1736, which lies on the distal end of the long arm of Chromosome 2 was found to be polymorphic and was genetically linked to the wrinkled trait, supporting this region as the location of *Se1*. The low marker diversity throughout the genome was consistent with the high degree of relatedness of the isolines.

An additional flanking marker, *Agt1,* was subsequently identified and mapped proximal to *Se1* and UMC1736. The physical interval between *Agt1* and UMC1736 based on the B73 reference is 1.26 Mbp in length. The two markers were used to screen a population of 820 F_2 seedlings (representing 1640 gametes) grown from phenotypically homozygous *se1*-type seeds selected on ears of self-pollinated F_1 plants. From these 1640 gametes, we identified 63 recombinants between these two markers based on presence of the *Se1* parental allele at UMC1736, *Agt1*, or both. These DNA samples were systematically evaluated with 15 additional markers to increase the mapping resolution (Table 2.3). This process narrowed *Se1* to an interval containing a single gene model, AC217415.3_FG004 (Figure 2.3). Three individuals remained heterozygous for the *Se1/se1* genotypes for all markers throughout the region, so they were excluded as the likely result of hetero-fertilization events (Sprague 1932).

A 24.3 kb region encompassing *Se1* and the nearest proximal and distal gene models was sequenced in both *Se1* and *se1* genotypes. A notable indel observed in this sequence was a 637

bp deletion in the *se1* genotype which completely eliminates predicted ORF AC217415.3_FG004 (Fig 2). A PCR marker for this indel perfectly cosegregated with the *se1* phenotype. Sequence data from the six closest crossover events identified from this group revealed that the crossover events closest to the deletion occurred between SNPs at 297 and 170 bp from its distal border, and between SNPs 197 and 39 bp from the proximal border of the deletion. This result confirmed that the deletion is the causal basis of the *se1* allele.

Bioinformatic analysis

In the B73 reference sequence, AC217415.3_FG004 contains a predicted open reading frame that is 173 amino acid residues in length, which would be translated from a GC-rich 522 bp sequence. Based on the gene model and ESTs available at www.MaizeGDB.org (EST GI: 31359437, 78025295, 5456061, & 5439303), the gene consists of only one exon with no introns. The predicted peptide is Glycine, Alanine, and Arginine-rich (36, 29, & 22 of 173 residues), and is 17.4 kD in mass. The *Se1* allele in the mapping population differs slightly from the B73 reference allele with several SNPs, two in-frame insertions and one inserted base that causes a frameshift near the 3' end, extending the length of the ORF to 555 nucleotides and 184 amino acid residues.

The AC217415.3_FG004 predicted protein does not have a high incidence of Serine or Threonine residues in the N-terminus, which would be indicative of a chloroplast or other plastid signaling peptide. TargetP rates the likelihood of chloroplast targeting and export *low*, mitochondrial destination *medium*, and other destinations to be *high*. ChloroP rates its chloroplast signaling as *medium-low* and Motif Scan results indicate a possible bipartite nuclear localization signal (RRVVFRAERDGGRLRLR), consisting of three Arginine and two Lysine

residues in an alternating fashion. However, there is only one part of a two-part signal so this does not appear to indicate that the putative SE1 protein is localized to the nucleus.

Homology

An analysis of the homology of *Se1* reveals several similar genes in *S. bicolor, O. sativa Japonica group, O. sativa Indica group, s. italica, T. aestivum, B. distachyon,* and *P. virgatum*, but none were found outside the monocots. *Sorghum bicolor* possesses the closest nucleotide match, Sb05g025625.1, with 64% coverage and a maximum identity of 90%. Most of these hits were identified in known syntenic regions, such as in rice, sorghum, and foxtail millet, and a comparison of putative homologs revealed two small conserved protein motifs. The first, "CTESLGSESGDVG", was found in all species except *P. virgatum*, while the second, "RAERRGGRLILT", was found in all alignments. None of these similar genes have any annotation information that indicates function.

Expression

The tissue-specific expression pattern of *Se1* was obtained with semi-quantitative RT-PCR performed on RNA from a range of B73 tissues (Figure 2.4). Expression of *Se1* in the reference B73 was low in whole seeds at 4 DAP, but increased at 8-12 DAP to a maximum at 16-20 DAP, and decreased slightly at 24 DAP. Expression in developing leaf tissue was observed in the base of a developing V5 leaf. Expression was low in root, seedling, internode, silk, and stem/SAM. Histone acetyltransferase complex component was identified as a suitable expression standard, and has consistent low expression in all tissues (Sekhon et al. 2011). cDNA gel band strength for *Se1* was similar to the standard, therefore *Se1* was a low-expressed gene overall. The

absence of a larger (250 bp) band including an intron in the expression standard lanes indicated the absence of DNA contamination.

Origin of se1

A set of ten sugary enhanced commercial and experimental sweet corn lines were genotyped with a codominant marker that detects the presence and absence of the *se1* deletion in both alleles. All sugary enhanced sweet corn lines genotyped possessed the same deletion identified in our mapping population, while B73 and a control non-sugary enhanced line Ia453*su* did not, as expected. IL677a and its progenitors were also genotyped, and the deletion was present in IL677a and IL44b, but not IL442a or Bolivia 1035.

2.5 DISCUSSION

The *se1* allele is due to a deletion of a predicted ORF based on sequence of a short, GC-rich gene near the distal end of the long arm of Chromosome 2. Our genetic mapping data excludes all other gene models in the region, giving us a high confidence that we have correctly identified the *Se1* gene. The presence of the *se1* deletion in ten sugary enhanced commercial lines tested supports this conclusion, and it should be designated the *se1* reference allele (*se1-ref*). The Corioco flour corn Bolivia 1035 was predicted to be the source of the *se1* allele (Schultz & Juvik 2004), so the identification of the deletion in IL44b was unexpected. However, IL44b derives from Narrow Grain Evergreen sweet corns (Gerdes et al. 1993), which maintain their eating quality longer than other varieties, and have a slower dry-down (Tracy, 1990), which are traits consistent with *se1*.

Starch metabolism in cereal endosperm is complex, and the overlapping functions of major enzymes are well known (Joen et al. 2010, Hannah & James 2008). In maize, the partial loss of ISA1 DBE activity in *su1* genotypes can be compensated for by pullulanase (ZPU1) DBE activity, and a *zpu1* null allele in combination with *su1* increases the magnitude of the high-WSP phenotype characteristic of *su1* (Dinges et al. 2003). Our isolines were homozygous for *su1* but had relatively high amylopectin and low WSP, therefore modifiers present in this genetic background likely partially compensated for the reduction in ISA1 activity. The phenotypic sensitivity of this unique genetic background to losses in starch debranching enzyme activity may assist the identification of additional genes in the starch pathway.

In these isolines, the *se1* deletion increased the proportion of WSP at the expense of amylopectin, indicating that *Se1* contributes to or complements the debranching activity of *SU1*. The putative SE1 protein may contribute to the function of *SU1* or a pullulanase independently or as part of a complex, and the absence of this protein may further limit debranching activity leading to higher total sugars and WSP and lower amylopectin. *Se1* has a similar expression pattern to *Su1*, albeit detected at lower levels in all tissues, which would put its expression in the appropriate developmental stages to play a role in this process.

Short peptides have been found to have regulatory roles in starch metabolism and other processes (Li et al. 2009), which provides the possibility that *Se1* could modulate the expression of other genes in the pathway. *Se1* is expressed in developing leaf and embryo tissue, and as early as 8 DAP in endosperm, compatible with a regulatory role in starch metabolism in these tissues. Important starch metabolic enzymes have been known to affect both biochemistry and have pleiotropic effects on other genes in the same or in different pathways. One proposed role

(Schultz & Juvik 2004) that appears to be excluded for the *se1* allele is directly increasing starch degradation, since the functional gene is absent in *se1* lines.

Presence-Absence Variation (PAV) describes the phenomenon where whole genes or genomic regions are present in one variety but absent in another, and has recently been found to be quite extensive in maize. It has been suggested that it may play a role in heterosis (Springer et al. 2009). However, few examples of PAV with significant phenotypic effects have been found (Swanson-Wagner et al., 2010). In *Arabidopsis*, PAV tends to be found for genes with low essentiality and result in minor impacts on phenotypes (Bush et al., 2014). Therefore, *se1* is a rare known example of PAV that can have dramatic effects on phenotypes while not being deleterious to plant development.

Our analysis indicates that *Se1* homologs are found throughout important grass species, and the absence of homology outside this group indicates that it could be a monocot-specific gene. Understanding the role of *Se1* could provide insight into the evolutionary divergence of metabolic processes since the last common ancestor of monocots and dicots. For instance, *Se1* could be a part of a suite of genes that evolved in grasses to adapt the quality or efficiency of dense energy stores in seeds and other tissues.

A better understanding of the genetic control of starch metabolism can lead to improvements in starch yield in grains that will be important for increasing the food supply to meet the world's future caloric needs. But it could also affect the cooking, baking, and eating quality of foods derived from starchy crops beyond the grasses. Already, varieties of crops are being developed with altered starch composition with the aim to improve digestive health (Slade et al. 2012). Insights from this research could be applied to other starch-rich crop species. For

now, the codominant marker we developed can be used to facilitate breeding high-quality sugary enhanced sweet corns.

2.7 REFERENCES

Azanza F, Klein BP, and Juvik JA. 1996. Sensory characterization of sweet corn lines differing in physical and chemical composition. *Food Sci.*, 1996 61: 253-257.

Bush SJ, Castillo-Morales A, Tovar-Corona JM, Chen L, Kover PX, Urrutia AO. 2014. Presence-absence variation in A. thaliana is primarily associated with genomic signatures consistent with relaxed selective constraints. *Mol Biol Evol*. 31(1):59-69.

Carey EE, Dickinson DB, and Rhodes AM. 1984. Sugar characteristics of sweet corn populations from a sugary enhancer breeding program. *Euphytica* 33 609-622.

Davis RC, Schadt EE, Smith DJ, Hsieh EWY, Cervino ACL, van Nas A, Rosales M, Doss S, Meng H, Allayee H, Lusis AJ. 2005. A genome-wide set of congenic mouse strains derived from DBA/2J on a C57BL/6J background. *Genomics*, 86(3): 259-270.

Doyle JJ, and Doyle JL. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12:13–15.

Ferguson JE, Dickinson DB, and Rhodes AM. 1979. Analysis of endosperm sugars in a sweet corn inbred (Illinois 677a) which contains the sugary enhancer (*se*) gene and comparison of *se* with other corn genotypes. *Plant Physiol.* 63(3):416-20.

Ferguson JE, Rhodes AM, and Dickinson DB. 1978. The genetics of sugary enhancer (*se*), an independent modifier of sweet corn (*su*). *J Hered* 69 (6): 377-380.

Gerdes JT, Behr CF, Coors JG, and Tracy WF. 1993. Compilation of North American Maize Breeding Germplasm. Crop Science Society of America.

Gonzales JW, Rhodes AM, and Dickinson DB. 1974. A new inbred with higher sugar content in sweet corn. *Hortscience* 9(1).

Gonzales JW, Rhodes AM, and Dickinson DB. 1976. Carbohydrate and enzymatic characterization of a high sucrose sugary inbred line of sweet corn. *Plant Physiol.* 58:28-32

Hannah LC, and James M. 2008. The complexities of starch biosynthesis in cereal endosperms. *Current Opinion in Biotechnology.* 19:160-165

James MG, Denyer K, and Myers AM. 2003. Starch synthesis in the cereal endosperm. *Current Opinion in Plant Biology.* 6:215–222.

Jeon J, Ryoo N, Hahn T, Walia H, and Nakamura Y. 2010. Starch biosynthesis in cereal endosperm. *Plant Physiology and Biochemistry* 48:383-392

Juvik, JA, Yousef GG, Han T, Tadmor Y, Azanza F, Tracy WF, Barzur A, and Rocheford TR. 2003. QTL influencing kernel chemical composition and seedling stand establishment in sweet corn with the *shrunken2* and *sugary enhancer*1 endosperm mutations. *J. Amer. Soc. Hort. Sci.* 128:864–875.

Li L, Foster CM, Gan Q, Nettleton D, James MG, Myers AM, and Wurtele ES. 2009. Identification of the novel protein QQS as a component of the starch metabolic network in Arabidopsis leaves. *Plant J.* 58(3):485-98.

Tadmor Y, Azana F, Han T, Rocheford TR and Juvik JA. 1995. RFLP mapping of the *sugary enhancer1* gene in maize. *Theoretical Applied Genetics* 91, 489-494.

Tracy, WF. 1990. Breeding for rust resistance in sweet corn. *Midwest Food Processors Association Processing crops manual and proceedings*. Dep. of Horticulture, Univ. of *Wisconsin*, Madison. p. 25-29

Tracy WF. 2001. Sweet Corn. In Hallauer A. R. *Specialty Corns*, Second Edition. Boca Raton, FL: CRC Press LLC.

Tracy WF. 2010. History, genetics, and breeding of supersweet (shrunken2) sweet corn. In: Janick J (ed) *Plant Breeding Reviews* (Vol 14), John Wiley and Sons, Inc, pp 189-236.

Tracy WF, Whitt SR, and Buckler ES. 2006. Recurrent mutation and genome evolution: example of *sugary1* and the origin of sweet maize. *Crop Science 46* (Suppl 1) S-49-S-54.

Schultz JA, and Juvik JA. 2004. Current models for starch synthesis and the sugary enhancer 1 mutation in Zea mays. *Plant Physiology & Biochemistry*. 42(6): 457-464.

Sekhon RS, Childs KL, Santoro N, Foster CE, Buell CR, de Leon N, and Kaeppler SM. 2012. Transcriptional and metabolic analysis of senescence induced by preventing pollination in maize. *Plant Physiol.* 159: 1730-1744

Sekhon RS, Lin H, Childs KL, Hansey CN, Buell CR, de Leon N, and Kaeppler SM. 2011. Genome-wide atlas of transcription during maize development. *Plant J.* 66(4):553-63.

Slade AJ, McGuire C, Loeffler D, Mullenberg J, Skinner W, Fazio G, Holm A, Brandt KM, Steine MN, Goodstal JF, and Knauf VC. 2012. Development of high amylose wheat through TILLING. *BMC Plant Biol*. 12:69

Sprague GF. 1932. The nature and extent of hetero-fertilization in maize. *Genetics* 17: 358–368.

Springer NM, Ying K, Fu Y, Ji T, Yeh CT, Jia Y, Wu W, Richmond T, Kitzman J, Rosenbaum H, Iniguez AL, Barbazuk WB, Jeddeloh JA, Nettleton D, and Schnable PS. 2009. Maize inbreds exhibit high levels of copy number variation (CNV) and presence/absence variation (PAV) in genome content. *PLoS Genet*. 5:e1000734.

Swanson-Wagner RA, Eichten SR, Kumari S, Tiffin P, Stein JC, Ware D, Springer NM. 2010. Pervasive gene content variation and copy number variation in maize and its undomesticated progenitor. *Genome Res.* 20(12):1689-99.

Table 2.1

Genotype	DAP	Year	Glucose	Fructose	Sucrose	Total Sugars	WSP	Starch	Total Polysaccharides	Total Carbohydrates
Se1	45	2006	12.7	12.6	34.3	59.6	137.0	423.1	560.1	619.7
Se1	45	2007	14.7	11.7	37.1	63.5	160.8	405.3	566.1	629.6
se1	45	2006	14.0	11.6	49.9	75.4	267.9	219.6	487.4	562.8
se1	45	2007	13.6	13.1	56.0	82.8	267.7	226.0	493.7	576.5
Se1	22	2011	46.7	48.4	62.5	157.6	154.0	350.1	504.0	661.6
se1	22	2011	53.5	52.2	77.1	182.7	263.9	194.0	457.9	640.6

Kernel Carbohydrates of mature, dry seed (45 days after pollination) and immature kernels at 22 days after pollination (DAP) for the *Se1* and *se1* isolines. Shown are the means of three samples from different ears for glucose, fructose, sucrose, total sugars, water-soluble polysaccharide (WSP), starch, total polysaccharides, and total carbohydrates. Units are reported as milligrams per gram of dry, lyophilized tissue. Total sugars were higher in *se1* compared to *Se1*, WSP was significantly higher, and starch was lower. The total polysaccharides and total carbohydrates were significantly lower in *se1* compared to *Se1*.

Table 2.2

Primer	Bin	Primer	Bin	Primer	Bin	Primer	Bin
unm1685	1.01	umc1947	2.08	umc2026	5.05	umc1799	7.05
bnlg1007	1.02	umc1992	2.08	bngl278	5.06	umc2190	7.06
bngl615	1.07	umc2005	2.08	bnlg1847	5.06	bnlg2037	8.01
phi094	1.09	umc2085	2.08	bnlg1306	5.07	phi119	8.02
umc1885	1.10	umc2202	2.08	bnlg1695	5.07	phi014	8.04
bngl131	1.11	bnlg1520	2.09	umc1225	5.08	phi121	8.04
phi064	1.11	bnlg1893	2.09	bnlg1043	6.00	bngl162	8.05
umc1605	1.12	umc1252	2.09	bnlg161	6.00	bngl666	8.05
umc1725	1.12	umc1256	2.09	umc1792	6.00	umc1384	8.07
umc1622	2.00	umc1551	2.09	bngl249	6.01	umc1279	9.00
umc2094	2.01	**umc1736**	**2.09**	bngl426	6.01	umc1647	9.00
umc1776	2.03	umc2184	2.09	umc1572	6.03	umc2128	9.02
phi109642	2.04	phi101049	2.10	nc010	6.04	umc2398	9.04
umc1658	2.06	umc2118	3.00	bnlg1443	6.05	umc1310	9.06
phi127	2.07	phi073	3.05	bnlg1617	6.05	umc1366	9.06
bnlg1169	2.08	bngl197	3.07	umc1859	6.06	umc1942	9.07
bnlg1316	2.08	bnlg1257	3.09	phi123	6.07	umc1277	9.08
bnlg1606	2.08	umc1288	4.02	umc1127	6.08	umc1239	10.00
bnlg1746	2.08	bnlg1126	4.03	umc2059	6.08	umc2399	10.00
bnlg1908a	2.08	bnlg1159	4.05	bnlg1642	7.00	umc1319	10.01
bnlg1940	2.08	bnlg1023	4.06	bnlg2132	7.00	umc2018	10.01
mmc0381	2.08	umc1651	4.07	mmp81	7.01	umc2034	10.02
npi298a	2.08	phi093	4.08	umc1159	7.01	umc2016	10.03
umc1230	2.08	umc2187	4.08	umc1270	7.01	umc1648	10.04
umc1464	2.08	bnlg1337	4.11	umc1632	7.01	bnlg1185	10.05
umc1516	2.08	umc1649	4.11	umc1428	7.02	umc1678	10.05
umc1604	2.08	bnlg105	4.12	bnlg1805	7.03	umc1061	10.06
umc1618	2.08	bnlg565	5.02	bnlg2271	7.03	umc1993	10.06
umc1633	2.08	phi008	5.03	bngl155	7.04		
umc1745	2.08	dupssr10	5.04	umc1407	7.05		
umc1798	2.08	umc1853	5.05	umc1760	7.05		

The *Se1* and *se1* isolines were genotyped at 121 SSR markers, arranged by maize chromosome bin locations. UMC1736 (bold) was the only marker in this screen that was identified as polymorphic between these two lines, and was linked to the *se1* trait.

Table 2.3a

Coord.	229,608,585	229,850,328	229,936,451	229,946,578	229,949,601	229,966,248	229,975,454	229,977,331	229,981,917	229,982,958	229,983,835	229,984,097	229,985,046	229,989,916	230,000,900	230,524,884	230,917,249	230,873,138
Marker Type	RFLP	SNP	SNP	GBS	GBS	RFLP	SNP	Indel	GBS	Deletion	RFLP	SNP	Indel	GBS	RFLP	SNP	RFLP	SSR
Marker Name	AGT1	ME 11	ME 61	ME 4	ME 68	MF 20	MG 12	MG 15	MF 29	MF 36F 30R DEL 2R	MF 3933	MF 39	MG 7757	ME 37	MD 27	MC 4	MA 15	UMC 1736
Individual																		
Se/Se control	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/se	Se/Se	Se/Se	Se/Se	Se/Se
se/se control	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
C 41	Se/se	Se/se	Se/se	se/se	se/se		Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
C 125	Se/se	Se/se	se/se	se/se		se/se	se/se	se/se	se/se	se/se		se/se		se/se		se/se	se/se	se/se
C 151	Se/se	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
C 152	Se/se	Se/se	se/se		se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se		se/se
C 159	Se/se	se/se	se/se	se/se		se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
C 160	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se		se/se
C 213	Se/se		se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se		se/se
22	Se/se		se/se	se/se	se/se	se/se	se/se			se/se	se/se		se/se	se/se	se/se	se/se	se/se	se/se
36	Se/se			se/se	se/se	se/se			se/se		se/se		se/se	se/se			se/se	se/se
49	Se/se			se/se	se/se	se/se					se/se	Se/Se	se/se	se/se	se/se		se/se	se/se
60	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
62	Se/se	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
129	Se/se		Se/se		Se/se	Se/se	Se/se	Se/Se	Se/se	se/se	se/se	se/se		se/se	se/se	se/se	se/se	se/se
184	Se/Se	Se/Se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
210	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se		se/se	se/se	se/se
250	Se/se	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
257	Se/se	Se/se	Se/se	se/se	se/se	se/se	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
297	Se/se	se/se	se/se	se/se	Se/se	Se/se	se/se	Se/Se	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
313	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
372	Se/se		se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se

Table 2.3a (cont.)

Coord.	229,608,585	229,850,328	229,936,451	229,946,578	229,949,601	229,966,248	229,975,454	229,977,331	229,981,917	229,982,958	229,983,835	229,984,097	229,985,046	229,989,916	230,000,900	230,524,884	230,917,249	230,873,138
Marker Type	RFLP	SNP	SNP	GBS	GBS	RFLP	SNP	Indel	GBS	Deletion	RFLP	SNP	Indel	GBS	RFLP	SNP	RFLP	SSR
Marker Name	AGT1	ME 11	ME 61	ME 4	ME 68	MF 20	MG 12	MG 15	MF 29	MF 36F 30R DEL 2R	MF 3933	MF 39	MG 7757	ME 37	MD 27	MC 4	MA 15	UMC 1736
Individual																		
Se/Se control	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/se	Se/Se	Se/Se	Se/Se	Se/Se
se/se control	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
506	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
522	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/Se	Se/Se	Se/Se
338†	Se/Se	Se/se	Se/se	Se/se	Se/se	Se/se	Se/se	Se/Se	Se/se	Se/se†	Se/se	Se/se	Se/se	Se/se	Se/Se	Se/se	se/se	se/se
394	Se/Se	se/se	se/se	se/se		se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
399	Se/Se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
400	Se/Se		se/se			se/se	se/se		se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
C 197	Se/se		Se/se	se/se	se/se	se/se	Se/se	Se/Se	se/se	se/se	se/se		se/se	se/se	se/se	Se/se	se/se	se/se
C 209	Se/se		Se/se	se/se	se/se	se/se	Se/se	Se/Se	se/se	se/se	se/se		se/se	se/se	se/se	Se/se	se/se	se/se
549	Se/Se	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se		Se/Se	Se/Se	Se/Se
550	Se/Se	Se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/Se	Se/Se	Se/Se
552†	Se/Se	Se/se	Se/se	Se/se	Se/se	Se/se	Se/se	Se/Se	Se/se	Se/se†	Se/se	Se/se	Se/se	Se/se	Se/Se	Se/Se	Se/Se	Se/Se
569	Se/Se		se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
576	Se/Se		Se/se	Se/se	Se/se	Se/se		Se/Se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se

Table 2.3b (cont.)

Coord.	229,608,585	229,850,328	229,936,451	229,946,578	229,949,601	229,966,248	229,975,454	229,977,331	229,981,917	229,982,958	229,983,835	229,984,097	229,985,046	229,989,916	230,000,900	230,524,884	230,917,249	230,873,138
Marker Type	RFLP	SNP	SNP	GBS	GBS	RFLP	SNP	Indel	GBS	Deletion	RFLP	SNP	Indel	GBS	RFLP	SNP	RFLP	SSR
Marker Name	AGT1	ME 11	ME 61	ME 4	ME 68	MF 20	MG 12	MG 15	MF 29	MF 36F 30R DEL 2R	MF 3933	MF 39	MG 7757	ME 37	MD 27	MC 4	MA 15	UMC 1736
Individual																		
Se/Se control	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se
se/se control	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
C 51	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se
C 106	se/se	se/se	se/se	se/se	se/se	se/se	se/se		se/se	se/se	se/se	se/se	se/se	se/se		se/se	Se/se	Se/se
C 173	se/se		se/se	se/se	se/se		se/se		se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se
C 201	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/Se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/Se	Se/se	Se/se	Se/se
2	se/se	se/se	se/se		se/se	se/se	se/se		se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se
10	se/se		se/se		se/se	se/se	se/se		se/se		se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se
50	se/se		se/se		se/se	se/se	se/se	se/se			se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/Se
79	se/se		se/se	se/se	se/se	se/se	se/se	se/se	se/se		se/se	se/se	se/se	se/se		se/se	se/se	Se/se
97	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se
104	se/se		se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se
107	se/se		se/se		se/se					se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se
110	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se
201†	Se/Se	Se/Se	Se/se	Se/se	Se/se	Se/se	Se/se	Se/Se	Se/se	Se/se†	Se/se	Se/se		Se/se	Se/Se	Se/se	Se/se	Se/se
202	Se/Se	Se/Se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/Se
213	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se
225	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se
248	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se
292	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se
304	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se

Table 2.3b (cont.)

Coord.	229,608,585	229,850,328	229,936,451	229,946,578	229,949,601	229,966,248	229,975,454	229,977,331	229,981,917	229,982,958	229,983,835	229,984,097	229,985,046	229,989,916	230,000,900	230,524,884	230,917,249	230,873,138
Marker Type	RFLP	SNP	SNP	GBS	GBS	RFLP	SNP	Indel	GBS	Deletion	RFLP	SNP	Indel	GBS	RFLP	SNP	RFLP	SSR
Marker Name	AGT1	ME 11	ME 61	ME 4	ME 68	MF 20	MG 12	MG 15	MF 29	MF 36F 30R DEL 2R	MF 3933	MF 39	MG 7757	ME 37	MD 27	MC 4	MA 15	UMC 1736
Individual																		
Se/Se control	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se	Se/Se
se/se control	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se
316	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se
318	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se
357††	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se	Se/se	Se/Se	Se/se	Se/se	Se/se
367††	se/se		Se/Se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se		Se/se	Se/Se	Se/se	Se/se	Se/se
381	se/se		se/se		se/se	se/se	se/se	se/se		se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se
411	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se
468	se/se	se/se		se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se	Se/se
469	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se
472	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se
508	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/Se	Se/se	Se/se	Se/se
509	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	se/se	Se/se	Se/se
514	se/se	se/se	se/se	se/se	Se/se	se/se	se/se	se/se		se/se	se/se	se/se	se/se		se/se	Se/Se	Se/Se	Se/se

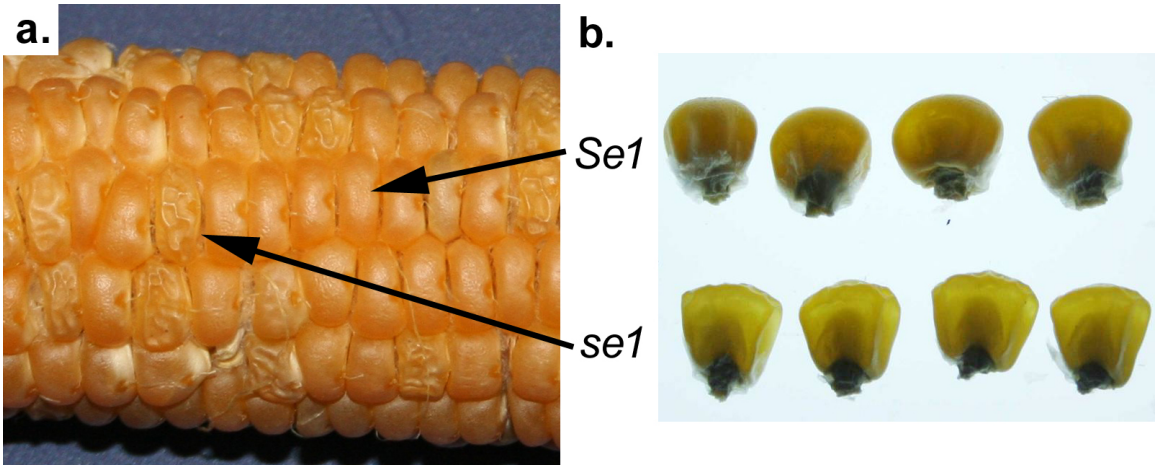
Supplemental Table 2.3a/b. Genotypes of recombinant plants grown from wrinkled seeds on selfed segregating *Se1/se1* heterozygous ears at markers used for genetic mapping. a. Genotypes begin as heterozygous Se/se on proximal marker AGT1, and progress to homozygous se/se at se deletion marker. b. Genotypes begin as heterozygous Se/se on distal marker UMC1736 and progress the same. Coordinates at top refer to the refgen_v2 B73 maize genome. RFLP, Restriction Fragment Length Polymorphism; SNP, Single Nucleotide Polymorphism; GBS, Genotyping By Sequencing; Indel, Insertion/Deletion. † Three individual plants genotyped as Se/se at all or nearly every marker, including deletion, suggesting that they are the result of hetero-fertilization, which were excluded from the final analysis. †† All wrinkled seeds genotyped from one ear shared identical genotypes through the region, suggesting recombination event occurred previous to this generation. These were counted as one individual in the analysis. Blank cells reflect genotypes that could not be determined after multiple attempts.

Table 2.4

Species	Chromosome	Locus name	Description	Synteny	Percent Match	Selected peptide match 1	Selected peptide match 2
Z. mays	2	AC217415.3_FG004	*Se1*	NA	100%	AVGACTESLGSESGDVG	RAERRGGRLILTEV
S. bicolor	5	Sb05g025625.1	No annotation	syntenic	60%	AVGACTESLGSESGDVG	RAERRGGRLILTEV
S. italica	1	Si027819m.g	PUF	syntenic	52%	CTESLGSESGDVG	RAERRGGRLILT
O. sativa Japonica group	11	LOC_Os11g42410.1	PUF	syntenic	47%	CTESLGSESGDVG	RAERRGGRLILTEV
B. distachyon	1	Bradi1g74990.1.0	No annotation	syntenic		GACTESLGSESGDVG	RAERRGGRLVLTEV
O. sativa Indica grp	6	OsI_22023	PUF	unclear		CTESLGSESGDVG	RAERRGGRLILTEV
O. sativa Indica grp	6	OsI_22021	PUF	unclear		CTESLGSESGDVG	RAERRGGRLILTEV
P. virgatum	Unmapped	Pavirv00027469m.0	No annotation	unclear		no match	RAERRGGRLILTEV
T. aestivum	Unmapped	CD889882	No annotation	unclear		CTESLGSESGDVG	RAERRGGRLILT

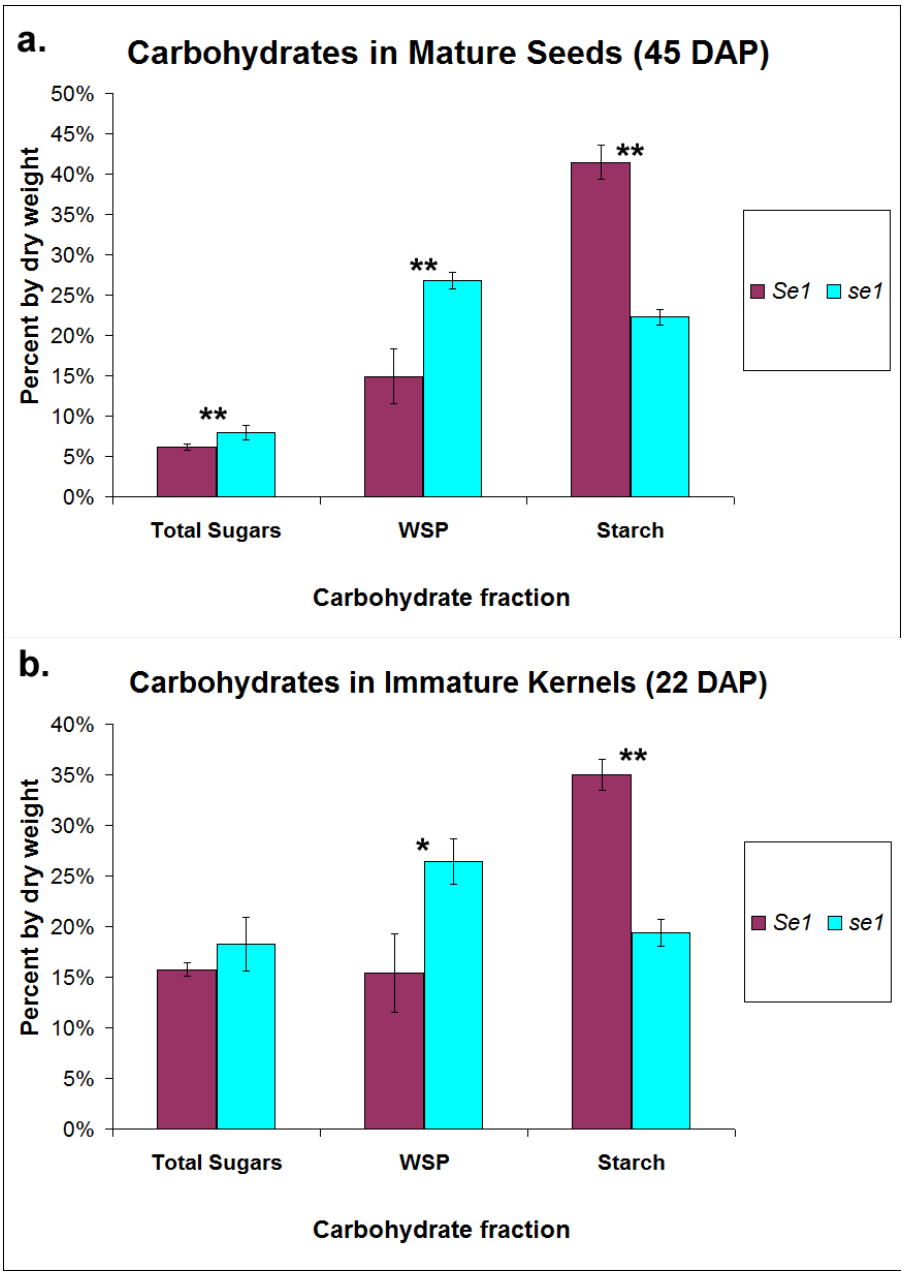
Family of *Se1* homologs, sorted by percent identity match to *Se1*, with currently available information about each locus. There was insufficient information available to calculate a percent match for several homologs, and no homologs had annotation information indicating their function, except when identified as a Protein of Unknown Function (PUF). A comparison of the predicted protein sequences of these homologs revealed two small motifs conserved in all but one homolog. The *S. bicolor* homolog Sb05g025625.1 had the greatest similarity, which extends beyond these two motifs (data not shown). No homologs were detected outside of the grasses or monocots, however there are few non-grass monocots that have been sequenced.

Figure 2.1



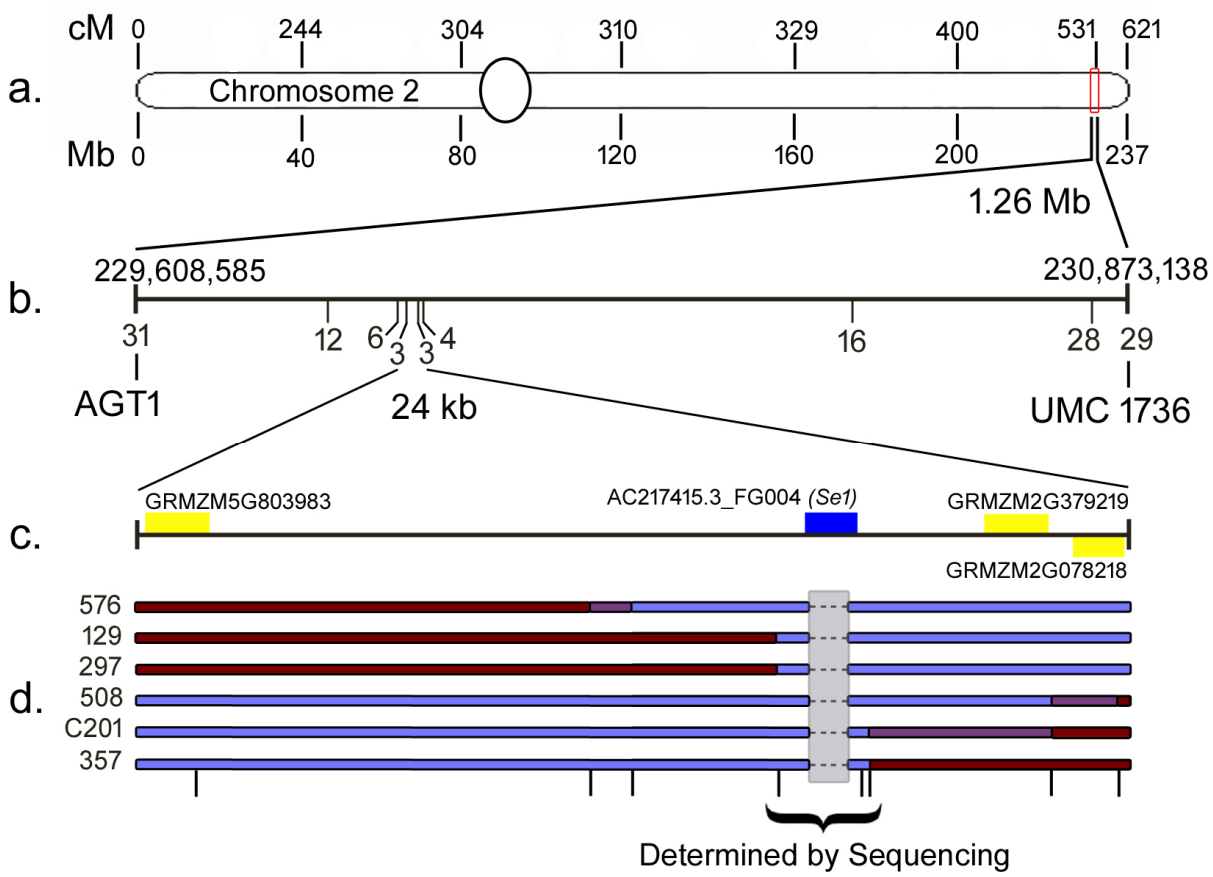
***Se1* and *se1* phenotypes in the mapping population and isolines. a. Segregating ear showing smooth *Se1* (homozygous and heterozygous) and wrinkled *se1* (homozygous) dry seeds. Note the lighter color of the wrinkled seeds, which is often associated with *se1*. b. *Se1* seeds are opaque when viewed on a lightbox, while *se1* seeds are translucent.**

Figure 2.2



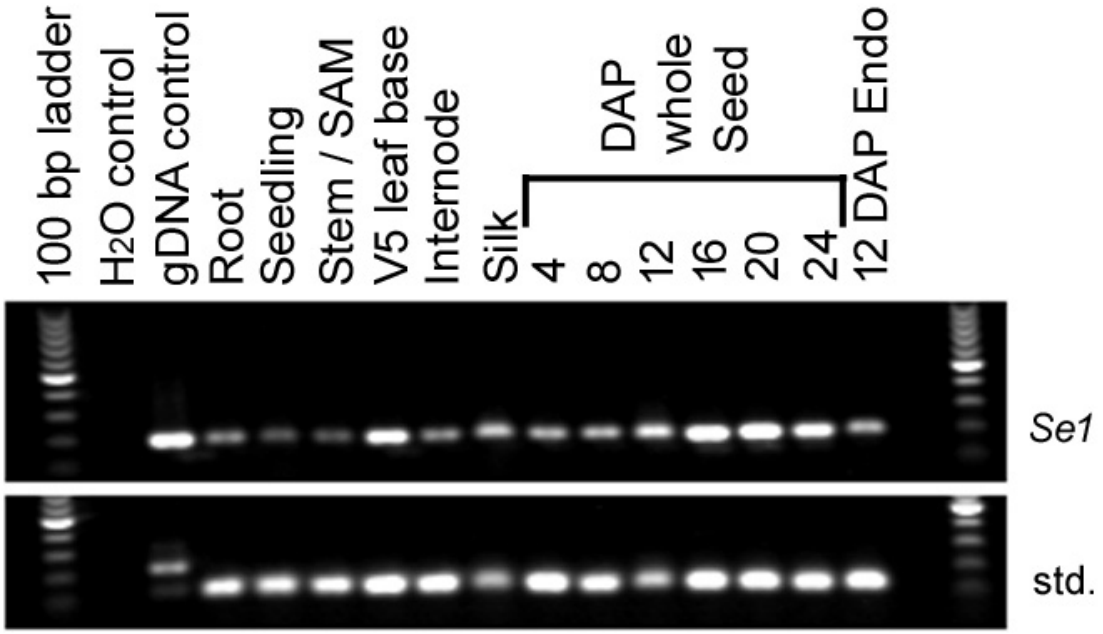
Carbohydrate analysis of mature seeds (a. 45 DAP) and immature kernels (b. 22 DAP) in *Se1* and *se1* isolines. Frozen and lyophilized whole kernels from three ears of each genotype were analyzed for total sugar, WSP, and starch content. The mature seeds from 2006 and 2007 were combined in the statistics, and there was only one year (2011) for the 22 DAP samples. Error bars are standard deviation. * 0.05 > p 0.01; ** 0.01 > p.

Figure 2.3



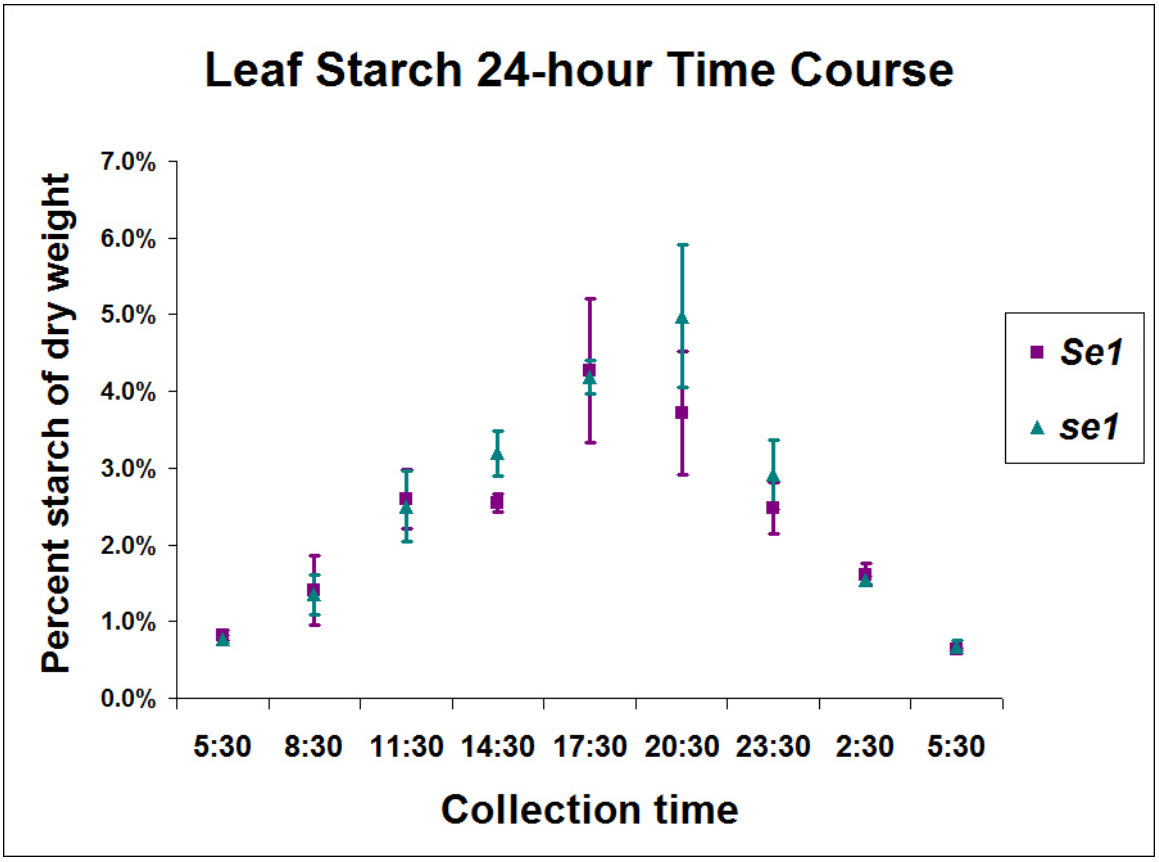
Genetic mapping of *se1*. a. Location of *Se1* region on chromosome 2 indicated by red box near the distal end. b. First zoom level of *Se1* region in B73 showing 1.26 Mb between the markers AGT1 and UMC 1736. Mapping markers and the number of recombinant plants with the *Se1* genotype from the mapping population are indicated by the vertical lines and associated numbers. c. Second zoom level showing 24 kb surrounding *Se1*, with gene models indicated by the blue and yellow bars. d. Mapping data from 3 proximal and 3 distal recombinants (identified at left). Vertical lines indicate marker locations. Red bar represents regions heterozygous for *Se1* and *se1* genotypes, light blue indicates *se1* homozygous genotype, and purple indicates unknown genotype between markers. The genotypes of recombinants at the *Se1* locus was determined by sequencing. All recombinants shown and homozygous *se1/se1* controls possessed the deletion indicated by the gray box and dotted lines, and all other gene models were excluded by the markers used for fine mapping.

Figure 2.4



Semi-quantitative RT-PCR of *Se1* compared to an expression standard in several tissues and developmental time points in B73. Expression was observed in the base of a developing leaf (sampled during the V5 stage), whole kernels from 12-24 DAP, and endosperm (12 DAP). Histone acetyltransferase complex component (GRMZM2G129817) was used as an expression standard for the same samples. The larger 250 bp band in the control gDNA lane includes a small intron, whose absence from the other lanes indicates negligible DNA contamination in these samples.

Figure 2.5



Mature leaf starch data from a 24-hour time course in the field, between *Se1* and *se1* genotypes. Means are indicated by symbols, and bars indicate one standard deviation from the mean. Time is in 24 hour notation, with sunrise at 5:30 and sunset at 20:30.

Acknowledgement of Co-authorship for Chapter 3

Manuscript Title: Development of educational videos to raise the profile of plant breeding and improve curricula.

Manuscript Authors: Karl J. Haro von Mogel, William F. Tracy, Shawn M. Kaeppler

Author contributions: WFT contributed to the conception and vision for the project and one of the interviews. SMK contributed to the conception and vision for the project, coordinated interviews, and supervised and reviewed the results in accordance with educational goals.

Chapter 3: Development of educational videos to raise the profile of plant breeding and improve curricula.

3.1 ABSTRACT

Plant breeding is an important human activity, and demand for trained plant breeders is increasing, but training for new breeders is lagging behind. Additionally, it is difficult for every plant breeding program to provide opportunities to become exposed to the methods used to breed other crop species. To address these issues, we have developed two series of videos to contribute to plant breeding education, called *Fields of Study* and *Pollination Methods.* The first is intended to raise the profile of plant breeding among high school and college students, and focuses on the human aspects of plant breeding and what plant breeders most enjoy about their profession. The breeders discuss what crops they work on, the importance and impact of their research, and their personal connection to their chosen career. The second series is intended for biology classrooms and consists of step-by-step demonstrations of how to make controlled crosses with different plants, and covers a wide range of techniques applicable to open-pollinated, insect-pollinated, and self-pollinated crops, including both agronomic and horticultural species. These twelve videos are available online, and have been well-received by educators and students alike.

3.2 INTRODUCTION

Humans have bred plants for thousands of years, and it is as important of an activity today as it was at the dawn of civilization. Plant breeding is the practice of changing the genetics of plants in order to produce desired characteristics (Sleper and Poehlman, 1995). Over many generations, the often slow process of generating variation and selecting plants with improved traits has transformed many species dramatically, such as the evolution of maize from the wild grass, teosinte (*Zea mays* ssp. *parviglumis*) (Wright et al. 2005). With a deeper understanding of underlying genetic processes, and with new tools and techniques, modern plant breeding has accelerated this process.

Today, professional breeders working for public and private institutions have a critical role in food security and quality of life by maintaining and improving the characteristics of important crops in the midst of constantly changing biotic and abiotic stresses from diseases to drought, and changing farming systems and consumer preferences. “Backyard” or hobbyist breeders also maintain and select unique phenotypes, such as heirloom tomatoes, showy flowers, and record-breaking pungent chili peppers. Some of the results of these hobby breeding efforts can also become economically and culturally important, and similar serendipitous backyard hybridizations have been important in the domestication of different species (Hughes et al. 2007).

The number of degrees awarded in plant breeding has been in decline for several decades (Guhner and Wehner, 2003). Public financial support has declined, and several institutions have reduced or eliminated their plant breeding programs altogether. The supply of new plant breeding graduates does not meet the demand, and industry is expecting that the number of plant breeders trained over the next 10 years will not be sufficient to meet their demand. Raising the

profile of plant breeding could help to attract students to pursue careers in this essential area. Innovative media approaches may reach students who might not otherwise become exposed to plant breeding. These same approaches could also assist in the training process in college classrooms.

Effective media outreach employs basic communication strategies, such as priming and framing. Priming is a media theory that says that a prior stimulus can affect how we think about or act in a later situation. Two neurological models have been proposed to explain this phenomenon, such as activation of memory through associated nodes, or calling up mental models that contain other ideas. One of the important aspects of priming is that it can be used to establish how people or issues are evaluated by new information (Bryant and Oliver, 2008). In practice, thinking about a particular subject can prime the mind to bring up other, related ideas at a later date.

Framing theory deals with how information about an issue is presented. Frames define problems, diagnose causes, make moral judgments, and suggest remedies. By highlighting certain pieces of information, making them more salient increases the likelihood that they will be remembered over other pieces of information. Even identical situations, if described differently, can lead people to different decisions about what is the best course of action. Frames can be useful to reduce the complexity of an issue into digestible portions that can mesh with already existing frames of reference in the audience (Scheufele and Tewksbury, 2007).

Multimedia combines different forms of media such as video, audio, and text, which can enable communication that is engaging to the audience (Tannenbaum, 1998). Combined with the ubiquity of internet-based connectivity, digital multimedia can be shared widely and rapidly. For

this reason, it can be an appropriate method to communicate information widely to target audiences to raise awareness and influence perceptions.

Multimedia has also been useful for conveying information in the classroom, with some students reporting that they were able to understand more complex concepts due to multimedia presentations (Nowaczyk et al., 1998). It is also well-received by most students and instructors (Krygier et al., 1997; Luna et al., 1997), and can be re-viewed with minimal effort, thus increasing the efficiency of instructors.

The advantage of multimedia for learning about specific scientific subjects is that it can provide a way to demonstrate abstract or complicated concepts in a more concrete form. One such abstract concept in plant biology and breeding is the biology of reproduction, and the techniques required to perform controlled pollinations. Students do not always have the opportunity to learn these details by directly working with plants, and even so, will likely not have access to the many different types of reproductive systems employed by different species.

With this in mind, we have developed two series of videos to contribute to plant breeding education. The first is a series of interviews with accomplished plant breeders, called *Fields of Study*. This series is intended to be a primer for interest in plant breeding. Thus the target audience is high school students and incoming college students. These videos are suitable for a general audience, and will also have educational value for members of the public.

Pollination Methods, consists of step-by-step, instructional videos on how to make controlled crosses with plants. The *Pollination Methods* videos are technically-oriented and are targeted to college students with some background in plant biology. They will also be useful for hobbyist and commercial breeders.

3.3 MATERIALS AND METHODS

Fields of Study

The *Fields of Study* videos were assembled from original interviews filmed with accomplished plant breeders that could speak to different aspects of plant breeding. These included commercial breeding, molecular aspects, taste, biofuels and environmental remediation, and breeding for developing countries. Each breeder was asked questions that addressed three central themes: What do they do as plant breeders, how do they select a few successful plants from diverse sources, and what do they enjoy about their career. Additionally, they were also asked about how they decided to become a plant breeder, and what kinds of skills and education are important for their career. The breeders were also asked to describe any familiar varieties of crops that they developed. This interview footage was reviewed and edited to emphasize these themes.

The *Fields of Study* series includes the following interviews:

- Sweet Corn Breeding with Bill Tracy
- Pepper Breeding with Molly Jahn
- Switchgrass Breeding with Ken Vogel and Michael Casler
- Watermelon Breeding with Xingping Zhang
- Apple Breeding with Jim Luby
- International Breeding with Kevin Pixley

Pollination Methods

The *Pollination Methods* videos were created with a different strategy. We selected crop species that represented different reproductive patterns, life cycles, and crossing techniques. Expert plant breeders were consulted on the biology and crossing techniques and considerations unique to each crop species, and were videotaped demonstrating those methods. Scripts were written to narrate each video (Appendix B), and were assembled in a side-by-side table format

for matching script segments to the visual elements that would edited into the video at that point. This strategy assisted with determining what footage, images, and graphics were necessary and facilitated communication between the producer and editor.

Each video covers one or two crop species, and explains the basic genetics of each crop, its origins and compatible wild relatives, and demonstrates effective crossing techniques. Each *Pollination Methods* video has a *Special Genetics* section added to the end of the video, to further explore a unique and interesting aspect of each crop, such as pungency in peppers, hybrid seed production in maize, and an explanation for how seedless watermelons are made. (Figure 3.2).

The *Pollination Methods* series includes the following videos:

- Corn
- Cucurbits (cucumber, melon, squash)
- Solanum (potato & tomato)
- Peppers
- Fruit trees
- Carrots & Beets

Video development

The content in the videos was obtained from diverse sources. Footage was primarily recorded with a full-size broadcast-quality Sony DV-cam camcorder with a macro-capable lens, which is important for zooming close to plant structures to show detail (Figure 3.1). Video footage taken abroad was obtained with a Canon GL2 DV-cam, which was more portable than the full-size Sony. Video footage was captured in a 4:3 aspect ratio. Interview audio was captured with a wireless lavalier microphone, which enabled high-quality sound both indoors and in the field. Voice-over audio was recorded with a broadcast-quality condenser microphone in a sound booth. Still photographs were captured with a Nikon D60 with an 18-55mm zoom

lens, and a macro lens was used for some close-up shots. Microscope photographs were taken with a digital camera mounted on a dissecting microscope.

While scientific sources of information provided adequate information for understanding breeding concepts such as polyploidy and wide crosses, it was necessary to generate visually interesting original graphics to illustrate them in video form. For instance, a *Capsicum* breeding complex diagram was generated to illustrate breeding relationships (Figure 3.3), and chromosome cartoons were made to illustrate polyploidy (Figure 3.2).

Editing was conducted at Merit Media at UW-Madison, formerly the Instructional Media Development Center (IMDC). Initially, video editing was done with Avid Express Pro. This changed to Final Cut Pro when Merit Media upgraded their system. Generally, each video went through three drafts: a rough cut, review cut, and final cut. The rough cut has the overall structure of the video assembled so that any major changes needed could be identified, and also indicates where additional still photos, diagrams, or video footage would be needed. The review cut is in near-final form so the precise details of the video's composition can be examined and the proper credits for content contained in the video can be written. The final cut has the credits added, and is the version that is to be released.

Since the videos are intended for wide distribution and availability for educators, students, and the general public, multiple channels for distribution are necessary. Social video sharing sites such as YouTube and Metacafe provide a convenient means for distribution to interested publics, with an acceptable loss in video quality for that end use. However, high video quality and portability are important for educators and breeders, who might find the ability to take the pollination methods videos to the field or greenhouse to be convenient for teaching and refining crossing techniques.

There is significant variation in video formats and digital filetypes in use today. Each method of video compression and file container has advantages and disadvantages in video quality, filesize, and player compatibility. A consideration of the end-use of a video is essential to deciding on a format that will meet intended objectives. Many social video sharing sites automatically convert uploaded videos into a format that will play properly on their sites. However for videos that are downloaded to users' computers, it is necessary to consider the benefits and drawbacks of different video formats.

Maintaining the video quality is important for displaying precise details of floral structure and demonstrating crossing methods, yet a small file size facilitates downloading to portable media devices. To compromise between these two objectives, the videos were initially produced in a QuickTime video format (MOV) in two sizes (640x480 and 320x240 pixels), encoded with the H.264 video codec that allows for good video quality at small file sizes. The benefit to this format was that it could be played on popular iPod and iPhone portable media players, and on both Apple and Windows-based computers with QuickTime. The drawback was that this format was not compatible with Microsoft PowerPoint, which is commonly used for classroom lectures.

As the videos were being produced, another video file format entered into wider use that allowed for more broad compatibility with Microsoft Windows Media Player and PowerPoint programs. The standard format for the videos produced in this project was switched to an M4V file container, encoded with the NTSC DV codec, at 480:360 pixel resolution. This resulted in an increase in file size relative to the smaller MOV format, but at a sizeable gain in video quality.

Fields of Study Evaluation

A pilot evaluation of the effectiveness of three Fields of Study videos was conducted on April 6, 2009 at Sauk Prairie High School in Prairie du Sac, Wisconsin. The Corn, Pepper, and Switchgrass breeding videos were shown to 16 students in an animal, plant, and soil science class. After watching the videos, the students were given a short questionnaire and asked to answer the following questions:

1. Did the videos interest you in plant breeding? Why or why not?
2. What do you think plant breeders do?
3. How is plant breeding important?
4. Is there much diversity with each crop? If so, how are they diverse?

After they finished the questionnaire, they were given time to talk about plant breeding, ask questions, and examine ears of corn segregating for different traits. Their responses to the questionnaire were analyzed for patterns.

3.4 RESULTS

These 12 videos are currently available to view or download at the University of Wisconsin Plant Breeding and Plant Genetics Program website at http://plantbreeding.wisc.edu/educators/videos/, and on YouTube at www.youtube.com/wiscplantbreeding/ and Metacafe at www.metacafe.com/channels/wiscplantbreeding/. They were promoted on the group science blog, Biofortified (www.biofortified.org/blog/), and picked up by other social media sites as a result.

The reception to these videos has been good online, as the total number of views for all videos on YouTube and Metacafe exceed 145,000. The distribution of the number of views is not equivalent for each video. Nearly half of the views (70.000) were for the corn pollination

methods video. The pepper pollination video garnered 25,000 views, and the cucurbit video, nearly 13,000. The Fields of Study videos each had 2,000-4,500 views. Via the UW-Madison PBPG website, approximately 6,000 total views and downloads had occurred as of 2011 (Plant Breeding and Plant Genetics Program website, accessed 12-15-2011), however the program website was moved to a different server and further tracking of these figures is currently not feasible.

Reviews by university faculty and graduate students have been positive, and the pollination methods videos were used in a plant breeding and biotechnology course for five consecutive years, and a horticulture course for two years. They have been presented at two scientific conferences, and feedback was used to improve the approach taken for subsequent videos.

Pilot Experiment Evaluation

The responses from the students who participated in the pilot experiment were positive, and gave insight into the value of the Fields of Study series. These will now be examined.

1. "Did the videos interest you in plant breeding? Why or why not?" In response to the first question, one quarter of the participants indicated that the videos made them interested in plant breeding. Of those who answered no, many indicated that they were not interested in plant sciences, and one student wrote that they wished there was more information on the education needed to become a plant breeder.

2. "What do you think plant breeders do?" Almost all of the answers to the second question indicated that they picked up on this information. They wrote that breeding and research can improve traits such as yield, environmental adaptation, and greater nutrition.

3. “How is plant breeding important?” Several students responded by mentioning the role of plant breeding in our food supply, including greater plant survival, a more abundant supply, and more nutritious food as important outcomes.

4. “Is there much diversity with each crop? If so, how are they diverse?” The answers to this question were more varied. Some of their responses did mention several traits such as size, color, taste, nutrition, yield, environmental adaptation, and drought tolerance. However, several responses, i.e. “each plant has its own way of helping the environment” indicated that they may not have understood this question very well, or that the videos were not clear about this point.

Indications of the effectiveness of the two video series have also come through personal communication from target audiences. Several backyard breeders have communicated their satisfaction with the Pollination Methods series, and two incoming plant breeding students have reported that the Fields of Study series influenced their decision to study plant breeding in graduate school.

Awards

Several videos in these series also won first and second-place awards in the Chlorofilms online plant biology video contest (www.chlorofilms.org).

3.5 DISCUSSION

Some studies indicate that multimedia presentations do not necessarily result in more learning or information retention when compared to more traditional forms of education (Krygier et al., 1997; Luna et al., 1997; Nowaczyk et al., 1998). This does not mean that multimedia is not beneficial, because it can increase the efficiency of instructors. Videos are no substitute for

hands-on learning experiences, and their use could be improved with plant biology lesson plans that involve breeding plants with short reproductive cycles, such as Wisconsin Fast Plants© (Williams and Hill, 1986) or other fast-cycling plants.

In the Plant Breeding and Biotechnology course, the importance of hands-on experience with plants to generate a firm understanding of the subject matter was apparent. By first teaching a lecture augmented with Pollination Methods videos, followed by a second lecture that combined videos with an in-class activity with different stations and a handout to facilitate the activity, the students became engaged in the learning process, and were able to apply general concepts from the videos to new plant species that were not specifically covered.

The number of online views achieved demonstrates the general appeal of these videos, but the preference toward certain videos is intriguing. Maize is an important crop in the United States and globally, and the high number of views for the corn pollination video could be explained by a general interest in corn, or it could indicate that the videos are being used in training, since the sweet corn breeding video was not similarly high in views. Peppers are a very popular plant for backyard breeders, which is a fact that could explain why the pepper methods video was the next highest in views. In fact, much of the feedback obtained through personal communication was from hobbyist pepper breeders. The special genetics section in the cucurbit video explained how seedless watermelons are made, which in turn could explain the appeal of this video. If true, it would indicate that these videos are useful for general knowledge.

The high school classroom evaluation provided several insights about the Fields of Study videos, the methods used to evaluate them, and also how to promote plant breeding among high school students. Reponses to the questionnaire helped identify which questions were not entirely clear to the students. This iterative process of testing and refining an approach is essential for

communication projects, and in this case has already identified a need for more information to help an interested student understand what educational path will lead to becoming a plant breeder.

While the videos generated some interest in plant breeding, there were more questions and greater enthusiasm when a hands-on discussion began, using the ears of maize that had kernels segregating for visually distinctive traits. Since this classroom experiment only tested the students after the videos and before the discussion, it may not reflect what those students ultimately took away from the experience. In practice, classroom presentations involving these videos should include a way for students to learn and discuss plant breeding on a more direct level. Similarly for online videos, hyperlinks that can lead to pages that allow viewers to more deeply investigate the subject are also advised.

Since it is difficult to test the effectiveness of video-based outreach, we can consider how each video managed to effectively use priming and framing. By exploring the story of the breeder behind different familiar varieties of crops such as apples and watermelons that consumers encounter in the store, the *Fields of Study* videos develop a new mental model associated with those varieties. Recognizing the same or similar varieties in the future would theoretically prime viewers to think about plant breeding. Also, the subjects important for becoming a plant breeder that were highlighted in each video could prime students to consider studying these subjects, even if they do not eventually study plant breeding.

The framing used in the *Fields of Study* series is particularly important. By both talking about what each breeder finds exciting about their profession, and showing different aspects of the daily lives of breeders, the videos make a compelling argument for considering plant breeding in terms of universal themes of discovery and excitement that appeal to a broad

audience. The focus of each interview was chosen to highlight different areas of plant breeding, from sweet corn to molecular biology, to making an impact internationally, to present frames that could be appealing to different audiences.

The self-reported influence of the *Fields of Study* series on two students' decision to pursue plant breeding is a promising development. One student communicated that the range of different exciting-sounding activities that plant breeders do, as explained in the videos, was particularly appealing. This student also reported that they had some hands-on exposure to making crosses with plants, which reinforces the need to couple these presentations with a tactile experience. These videos should be disseminated widely to encourage more students to consider plant breeding as a career.

Expanding the number of students interested in crop improvement can help fuel an increase in plant breeders, and these two series may prove useful in promoting plant breeding as a career and educating future plant breeders. However, graduate programs are currently turning qualified applicants away for lack of funding. To be effective, these and other outreach efforts must also be backed up with a long-term increase in financial and institutional support to train new students at the graduate level.

3.6 REFERENCES

Bryant K, and Oliver M. B. (Eds.) 2008. *Media Effects: Advances in Theory and Research.* Third Edition, Mahwah, NJ: L. Erlbaum.

Chlorofilms, All Session Winners. Accessed 12-19-2011. http://www.chlorofilms.org/

Guhner N, and Wehner TC. 2003. Survey of U.S. land-grant universities for training of plant breeding students. *Crop Sci*. 43:1938-1944.

Hughes CE, Govindarajulu R, Robertson A, Filer DL, Harris SA, Bailey CD. 2007. Serendipitous backyard hybridization and the origin of crops. *PNAS* 4;104(36):14389-94.

Kreisel K. 2003. Evaluation of a computer-based nutrition education tool. *Public Health Nutrition*: 7(2), 271–277

Krygier JB, Reeves C, DiBiase D, and Cupp J. 1997. Design, Implementation, and Evaluation of Multimedia Resources for Earth Science Education. *Journal of Geography in Higher Education.* 21:1 pp. 17-39

Luna CJ, Mckenzie J. 1997. Testing Multimedia in the Community College Classroom. *T H E Journal (Technological Horizons in Education),* Vol. 24

Nowaczyk RH, Santos LT, Patton C. 1998. Student Perception of Multimedia in the Undergraduate Classroom. *International Journal of Instructional Media*, Vol. 25

Plant Breeding and Plant Genetics Program, University of Wisconsin-Madison. Plant Breeding Educational Videos. Accessed 12-15-2011. http://plantbreeding.wisc.edu/educators/videos/

Scheufele DA, and Tewksbury D.2007. Framing, Agenda Setting, and Priming: The Evolution of Three Media Effects Models. *Journal of Communication* 57 9-20

Sleper and Poehlman, 1995. *Breeding Field Crops*. Ames, IA: Wiley-Blackwell.

Tannenbaum RS. 1998. *Theoretical foundations of multimedia*. New York: Computer Science Press.

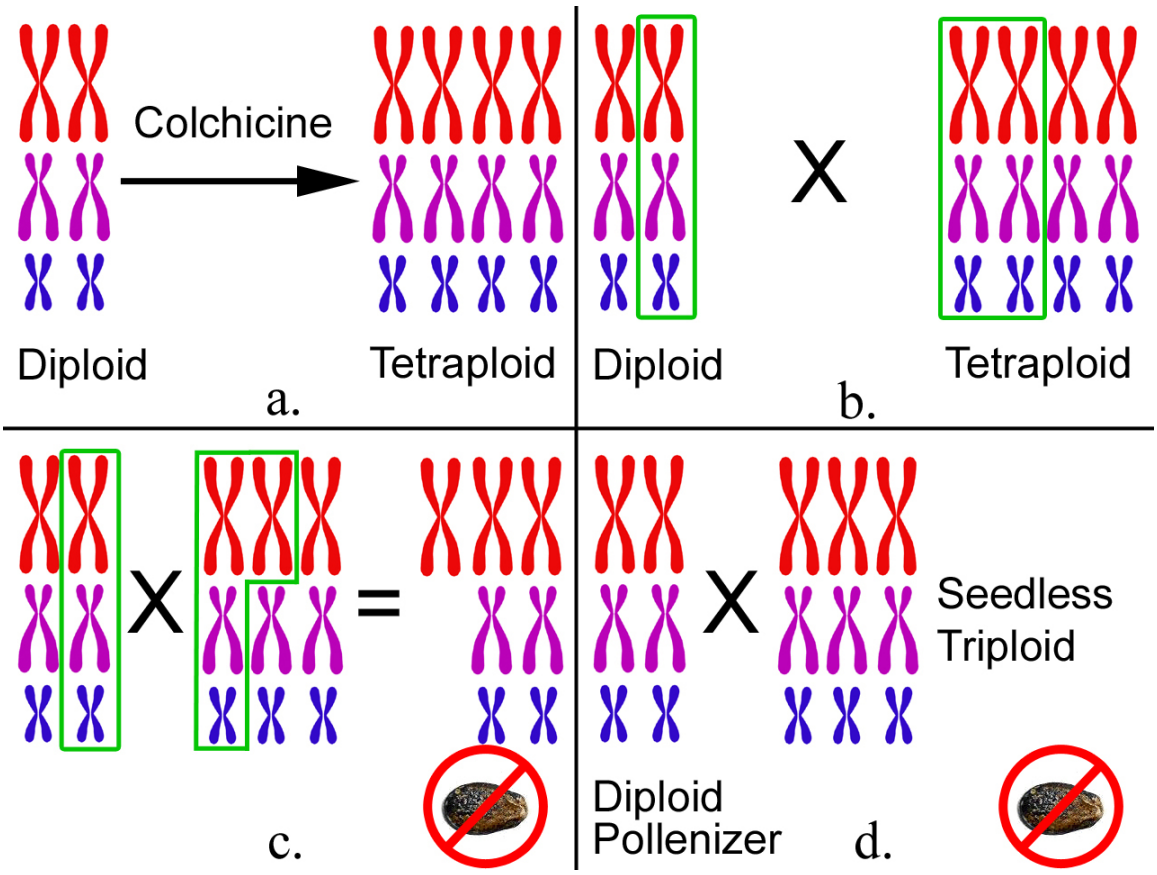
Williams PH, and Hill CB. 1986. Rapid-cycling populations of Brassica. *Science*. 232:1385–1389. doi: 10.1126/science.232.4756.1385.

Wright SI, Vroh I, Schroeder SG, Yamasaki M, Doebley JF, McMullen MD, and Gaut BS. 2005. The Effects of Artificial Selection on the Maize Genome. *Science* 308 (5726), 1310-1314.

Figure 3.1

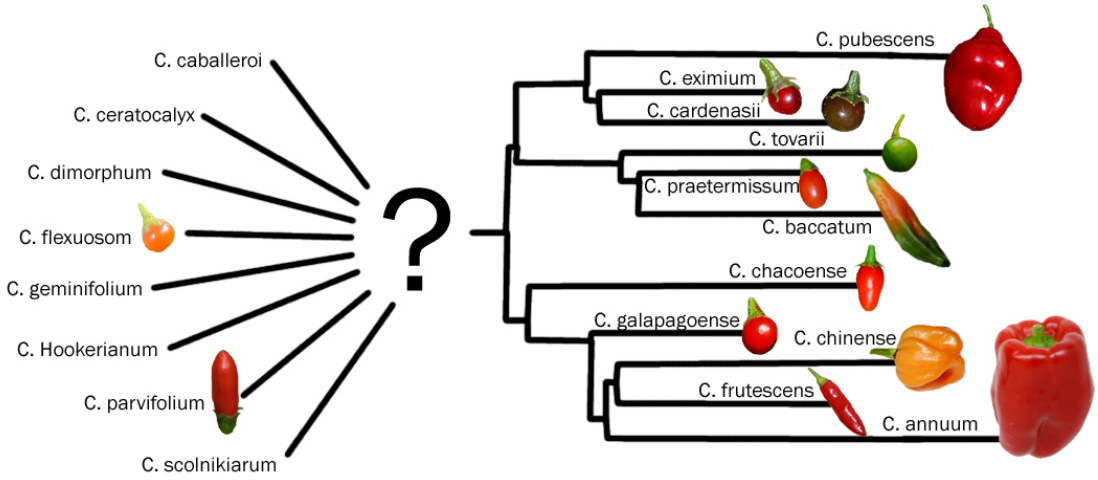
Videotaping in maize nursery at the West Madison Agriculture Research Station (WMARS). Filming on location in the field required pre-planning for weather and flowering conditions, and understanding the shots that needed to be filmed and photographed for future editing. When there are no more plants flowering there is little chance of obtaining pick-ups.

Figure 3.2



Four selected graphics from a sequence generated to explain the process of developing and growing seedless triploid watermelons. a. Chromosome doubling with colchicine to develop a tetraploid. b. Crossing a diploid and tetraploid to develop a triploid. c. Diagram of chromosome sorting showing the seedless nature of the triploid. d. Demonstrating the combination of diploid pollenizer and triploid seedless watermelons when grown by a farmer in the field.

Figure 3.3



Interrelationships between *Capsicum* species showing typical phenotypes and known relationships between and among the three *Capsicum* complexes (right), and uncertain relationships to other important members of the *Capsicum* genus. *C. flexuosum* and *C. parvifolium* are noted as bridge species between the complexes (left). These details will help assist with determining what breeding stratgegies can lead to fertile progeny for both professional breeder training and for hobbyists for whom this information may be difficult to find.

APPENDIX A

"STIPPLE": INITIAL GENETIC AND PHENOTYPIC ANALYSIS OF A POTENTIAL NEW STARCH PATHWAY MUTANT DERIVED FROM EMS MUTAGENESIS.

An experiment was conducted in 2010 to identify new recessive mutant alleles for *Sugary enhancer1* (*se1*). Approximately 3000 homozygous *Se1* seeds were treated with EMS according to the protocol in *Mutants of Maize* (Neuffer, Coe, & Wessler, 1997) and were planted alongside 1500 untreated near-isogenic homozygous *se1* seeds in alternating rows (Two rows of *Se1* M1 seeds for each row of *se1* seeds). The seeds were planted early so that the pollination period would be temporally isolated from other varieties of maize in the field. The M1 plants were de-tasseled before pollen was shed, therefore the majority of viable pollen that reached the *Se1Se1* ears came from the *se1se1* tassels in this plot, which was confirmed by inspecting random ears on *se1se1* plants for aberrant kernel phenotypes. Ears from plants containing new mutant alleles (*se1**) for Sugary enhancer would show a 1:1 segregation between the smooth phenotype of *Se1/se1* kernels and the wrinkled phenotype of *se1/se1** seeds. Due to the mosaic nature of plants treated with EMS as seeds, only a sector of an ear would be expected to show this pattern of segregation, and wrinkled seeds were expected to contain one copy of the already existing *se1* allele and one new recessive allele.

Figure A.1.

Figure A.1. Mutant A ear (rotated) showing normal Se1 seeds on most of the ear, and a region with segregation between smooth and wrinkled seeds. A portion of the ear decayed due to moisture in the field before harvest.

One ear with a sector with the expected segregation was obtained from this experiment (Figure A.1.), designated mutant A, along with several that were considered less likely candidates due to segregation throughout the ear. Several seeds from this ear were planted in the greenhouse and genotyped, sequenced, and the plants were self-pollinated. Two seeds were viable and both plants produced ears. Genotyping revealed that these plants were heterozygous for the deletion characteristic of *se1*, however sequencing revealed no new mutations within the known coding region of the gene. The self-pollinated ears were found to be segregating for the smooth and wrinkled phenotypes, indicating that a new *se1* allele was not obtained.

On closer examination of the ratio of smooth to wrinkled seeds on these ears revealed that the segregation pattern did not fit the expected ratio for a single gene (3:1), suggesting that there was a new, heritable mutation segregating in the seeds. Moreover, there seemed to be some phenotypic differences among the wrinkled seeds that suggest that a new mutation in another gene may have given rise to a unique phenotype. The wrinkled phenotype of homozygous *se1/se1* seeds in this background typically appears as sharply wrinkled and shrunken, with a lighter, more yellow color when compared to the smooth golden seeds of the *Se1* genotype. Some wrinkled seeds on this self-pollinated ear appeared to have a phenotype that has more starch fill in the base of the kernel and a more subtle "stippled" wrinkle pattern on the top of the kernel. Finally, not all wrinkled seeds fit into either of these two categories and instead appeared to have an intermediate phenotype. High-resolution photographs were taken of these seeds, and their phenotypes can be seen in Figure A.2. For the purposes of this analysis and for identification in the near term, the name "*stipple*" will be used for this new phenotype and the potential new gene that may be identified upon further study. A more detailed discussion of naming considerations will be made below.

Figure A.2.

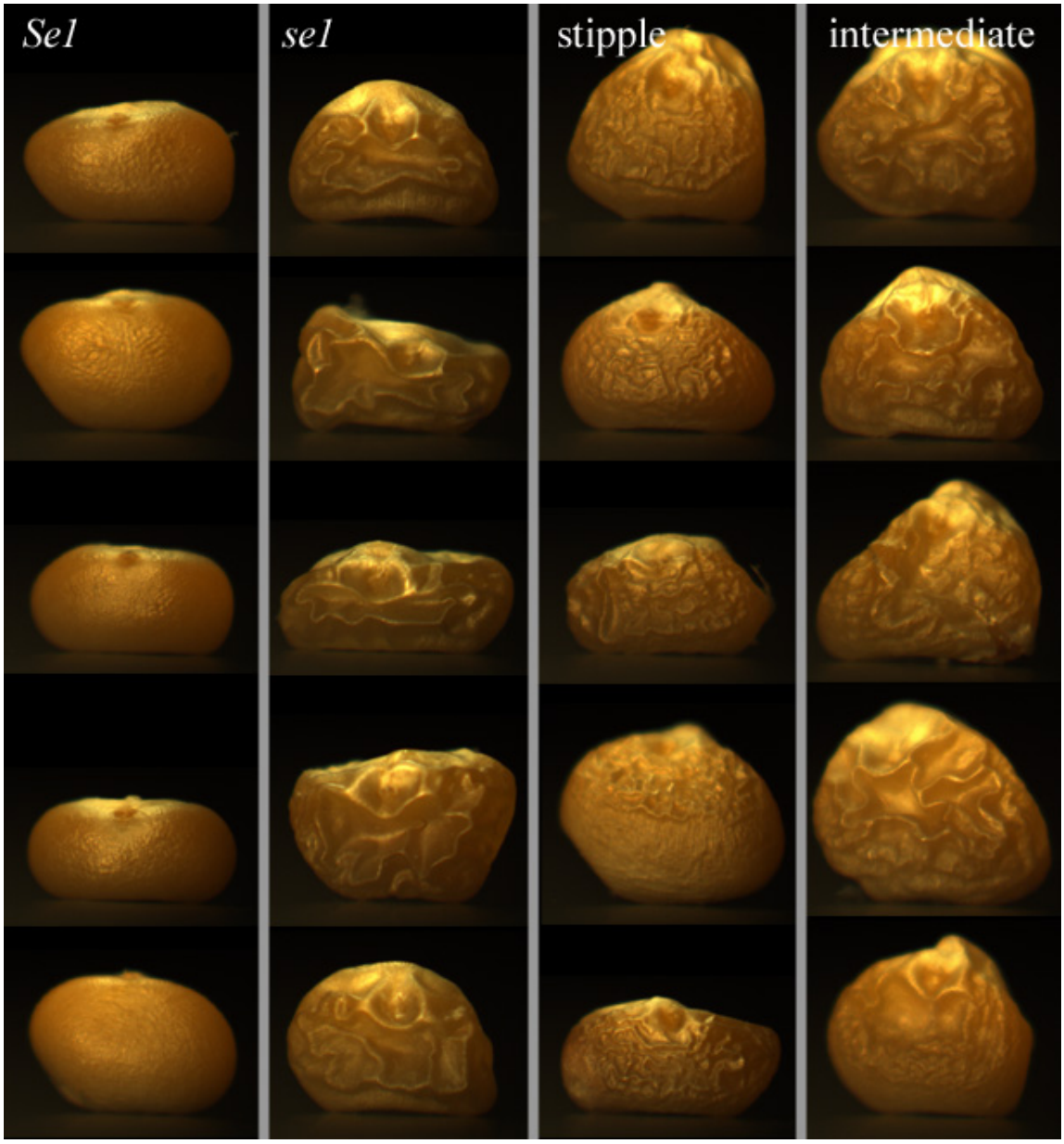


Figure A.2. A mosaic of high-resolution photographs taken of the tops of the seeds on the ear of a self-pollinated *Se1* M1 x *se1* plant, showing the different phenotypes observed in the seeds. Typical smooth-seeded *Se1* and deeply-wrinkled *se1* seeds are in the first two columns. The third column shows the "stipple" phenotype, and the fourth contains seeds that show a phenotype that intermediate between *se1* and stipple seeds.

Re-examining the original mutant A ear with these phenotypic categories as a rubric revealed that the wrinkled seeds resemble the stipple and intermediate phenotypes more closely

than a typical *se1* seed. However, an examination of *Se1* and *se1* ears harvested from multiple years revealed that there is some variation in how the *se1* phenotype appears on each ear, which may be due to differences in weather between years, and pollination and the timing of when the ears reach full maturity. This, combined with the intermediate phenotypes, makes it difficult to clearly demarcate between *se1* and *stipple* phenotypes in these ears. However, given that it appears to be a mutation in a second gene, it is possible to evaluate hypotheses about the mode of inheritance of *stipple*, and speculate about its biochemical role in producing this phenotype.

Mode of Inheritance

The initial mutant ear contains a sector of segregating seeds with an unknown boundary and some fungal damage, so the ratio of wrinkled to smooth seeds can only be estimated. Approximately 62 wrinkled seeds are observable with 66 smooth seeds between them, which suggests that they are segregating with a 1:1 ratio.

In the 2 self-pollinated *Se1* M1 x *se1* segregating ears, although there is some apparent difference in wrinkled phenotypes, it is difficult to place them in separate phenotypic categories, so they are currently only being counted as smooth and wrinkled. One ear also has fungal damage and is difficult to score, however there are approximately 73 smooth seeds and 67 wrinkled seeds. On the second, larger ear, most seeds were easily identifiable, with 67 smooth and 85 wrinkled. In total, 140 smooth seeds and 152 wrinkled seeds were scored. This is very close to a 1:1 ratio. (Chi = 0.49) Four hypotheses that may explain this non-mendelian segregation ratio will be explored to determine the likely mode of inheritance of *stipple*:

1. Dominant non-dosage dependent (independent assortment)
2. Additive linear dosage dependent (independent assortment)

3. Recessive mutant in a maternally imprinted gene (independent assortment)
4. Recessive mutant in a paternally imprinted gene (independent assortment)

Hypothesis 1,2: Dominant non-Dosage Dependent and Additive Linear Dosage Dependent

For both of these hypothetical gene actions, the phenotypes of the parent ear (Figure A.1) can be used to infer about the expected phenotypes of the selfed ears.

If the new mutation caused a dominant allele to emerge that gives rise to a similar phenotype as se, to be referred to as *Stipple* (*Stp*), this would produce a 1:1 segregation in the parent ear, as only the Stp___ genotype would be expressed as a wrinkled phenotype. In the selfed double heterozygous *Stpstp/Se1se1* ears, however, the kernels would be predominantly Wrinkled in approximately a 3:13 ratio, with only *stpstpSe1__* kernels appearing smooth (Table A.1). This does not agree with the observed 1:1 segregation ratio. (Chi = 163.4)

If the new mutation is additive with se, a double-heterozygous seed could give rise to a wrinkled phenotype as in the parent ear. This new allele will be referred to here as *stp*. Similar to the dominant mutation type considered above, this would also give rise to mostly wrinkled seeds in a 5:11 or 6:10 ratio, depending on the phenotype of *stpstp/Se1Se1* seeds, which have not been observed in the parent ear. In either case, 5:11 (Chi = 37.9) and 6:10 (Chi = 13.6) is still significantly different from the observed ratio (Table A.1).

Table A.1.

	Relative frequency of genotype	**1. Dominant, non-Dosage Dependent**	**2. Additive Linear Dosage Dependent**
StpStp/Se1Se1	1	Wrinkled	Smooth
StpStp/Se1se1	2	Wrinkled	Smooth
StpStp/se1se1	1	Wrinkled	Wrinkled
Stpstp/Se1Se1	2	Wrinkled	Smooth
Stpstp/Se1se1	4	Wrinkled	Wrinkled
Stpstp/se1se1	2	Wrinkled	Wrinkled
stpstp/Se1Se1	1	Smooth	Wrinkled or Smooth
stpstp/Se1se1	2	Smooth	Wrinkled
stpstp/se1se1	1	Wrinkled	Wrinkled
Phenotypic ratio		3:13 S:W	5:11 or 6:10 W:S

Table A.1. Side-by-side evaluation of dominant non-dosage dependent and additive linear dosage dependent hypotheses according to their expected frequencies when segregating independently.

The triploid nature of maize endosperm complicates analysis of this mutant under the dosage model given the limited amount of data obtained about specific genotypes, and the above analysis does not take this into account. The only kernels that have been observed where the endosperm is known have the following genotypes: (***Smooth***/*Wrinkled*) ***StpStpStpSe1Se1Se1***, ***StpStpStpSe1Se1se1***, ***StpStpStpSe1se1se1***, *StpStpStpse1se1se1* (all genotypes on segregating *Se1/se1* ears) and *StpStpstpSe1se1se1* (on the first mutant ear).

Genotypes involving more than one copy of the new mutant allele have not been observed, so it is difficult to know precisely what segregation ratios to expect. If each recessive

allele in both genes contributes an equal amount toward the wrinkled phenotype, and in both of the wrinkled genotypes above there are three recessive alleles, then we could assume that three recessive alleles in either gene confers a wrinkled phenotype. The results of this combination are below, with phenotypes indicated.

Table A.2.

Female and Male gametes	***StpStpSe1Se1***	***StpStpse1se1***	***stpstpSe1Se1***	***Stpstpse1se1***
StpSe1	***StpStpStp Se1Se1Se1***	***StpStpStp Se1se1se1***	***Stpstpstp Se1Se1Se1***	*Stpstpstp Se1se1se1*
Stpse1	***StpStpStp Se1Se1se1***	*StpStpStp Se1se1se1*	*Stpstpstp Se1Se1se1*	*Stpstpstp Se1se1se1*
stpSe1	***StpStpstp Se1Se1Se1***	*StpStpstp Se1se1se1*	*stpstpstp Se1Se1Se1*	*stpstpstp Se1se1se1*
stpse1	***StpStpstp Se1Se1se1***	*StpStpstp Se1se1se1*	*stpstpstp Se1Se1se1*	*stpstpstp se1se1se1*

Table A.2. Punnett Square analysis of triploid endosperm genotype combinations to determine the expected segregation ratio of additive linear dosage dependent hypothesis. Endosperm genotypes expected to produce smooth phenotypes are in bold, while genotypes with wrinkled phenotypes are designated with non-bold italics.

As it can be seen, the expected segregation ratio is 6:10, which is still significantly different from what has been observed (Chi = 13.6). If the contributions of alleles in each gene are significantly different, then other segregation ratios may be expected, so this ratio only works given the above assumptions.

Hypothesis 3: Recessive mutant in a maternally imprinted gene

In maternal imprinting, the copy of the gene inherited through the egg cell is silenced, while the paternal copy is expressed. This means that no matter what mutation may have showed up in a maternally imprinted gene on the parent ear, the phenotype would be determined by the paternal source, which was not mutagenized. A segregating phenotype would not be expected if a new mutation was generated in a gene that is silenced through the maternal side, although it may show up in the following generation. But since segregation was observed in the parental ear then this is not a likely explanation of the segregation ratio.

4. Recessive mutant in a paternally imprinted gene

In paternal imprinting, the copy of the gene inherited through the paternal line is instead silenced. Therefore the functional allele is from the maternal side. If a new recessive mutation was generated in a maternally imprinted gene in this screen, then it would manifest in the parental ear as a 1:1 segregation, as observed. Analyzing the F_2 seeds of the initial cross will require a Punnett Square (Table A.3)

Table A.3.

Female and Male gametes	***Stp$_i$Se1***	***Stp$_i$se1***	***stp$_i$Se1***	***stp$_i$se1***
StpSe1	***Stp$_i$StpSe1Se1***	***Stp$_i$StpSe1se1***	***Stpstp$_i$Se1Se1***	***Stpstp$_i$Se1se1***
Stpse1	***Stp$_i$StpSe1se1***	*Stp$_i$Stpse1se1*	***Stpstp$_i$Se1se1***	*Stpstp$_i$se1se1*
stpSe1	***Stp$_i$stpSe1Se1****	*Stp$_i$stpSe1se1*	***stp$_i$stpSe1Se1****	*stp$_i$stpSe1se1*
stpse1	*Stp$_i$stpSe1se1*	*Stp$_i$stpse1se1*	*stp$_i$stpSe1se1*	*stp$_i$stpse1se1*

Table A.3. Punnett Square analysis of paternal imprinted gene hypothesis. "*Stp*" is designated as the normal allele of the mutant gene, and *stp* as the mutant allele. Silenced copies of either allele will be designated with an 'i' subscript. Combinations marked in bold would show a smooth phenotype, italics, a wrinkled phenotype, and bold italics* are uncertain. This is considering that the wrinkled phenotype can arise through either homozygosity for *se1*, or a combination of recessive stp mutations and/or silenced copies of *Stp*.

Since homozygous *stpstp/Se1Se1* kernels have not been observed, it is not clear whether the two seed genotypes denoted with an asterisk would be either smooth or wrinkled. These two options produce segregation ratios of 6:10 (Chi = 13.6) and 1:1 (Chi = 0.49). The 1:1 segregation option is the closest to the observed ratios, meaning that from this data alone the likeliest explanation is that a new mutation has been obtained in a paternally imprinted gene (assuming that *stpstp/Se1Se1* seeds are not wrinkled).

Biochemical Considerations and Gene Function

The analysis of this new mutation is still in its early stages, so little can be concluded about its biochemical function. However, some of the phenotypic markers of this mutant suggest some mechanisms that can be taken into consideration for future research. The mature seeds

show an intriguing combination of traits. The fine wrinkles observed on the top of the kernels may indicate an inability to fully produce and store starch in the endosperm, which suggests a mutation in a starch metabolism gene. However, the presence of what appears to be plumpness at the base of the kernel could indicate that the loss of starch is not very severe. The new mutation could be in a gene that codes for a protein expressed late in endosperm development, and so the loss of this function would only slightly impair starch production.

The function of the potential new gene that this mutation occurred in may also be suggested by the characteristics of the line it was identified in. This line was selected to mendelize the *sugary enhancer1* trait, and may be sensitive to losses in starch debranching (DBE) activity, which would produce wrinkled in the mature seed. The new mutation could lie in or near a gene that is also involved in this pathway in maize endosperm.

Finally, there is the possibility that this mutation may be present in a gene that has already been described. For instance, it could be a mutation in *sugary1* that leads to a more severe reduction in function than the recessive allele present in these stocks.

Naming Considerations

The name *stipple* was chosen due to the similarity of the phenotype to a stippling brush pattern in wet paint, however, the term “stippling” is already in use to describe a phenotype with pigments appearing in small dots on the surface of the seed. In particular, the R-stippling mutant system is a well-described phenomenon in maize (Williams et al. 1984). Naming this new mutant *stipple*, though it can be considered descriptive of the seed phenotype could generate confusion in the literature. “Spackled” or “Spackling” could also describe the phenotype, and a *spackle*

(*spk*) name is unreserved. Alternate names will be considered, based on other phenotypic markers.

It currently remains uncertain whether seeds in this genetic background that are homozygous for the new mutation but homozygous for *Se1* are wrinkled or smooth, or display a phenotype that is distinct from either. The wrinkled seeds identified on the mutant A ear were heterozygous for *se1*, which seemed to reveal the presence of the new mutation, and conversely the new mutation can be seen as revealing the presence of one copy of *se1*. It may be found that a single *se1* allele is required to observe this phenotype, which although it seems unlikely it might suggest a more poetic name such as *sugary enhancer revealer* (*ser*).

A name based on biochemical function may be more useful and less confusing. Following further study, if the phenotype of ears homozygous for this mutation causes an increase in sugar content and maintains phytoglycogen similar to *sugary enhancer1*, then it may be appropriate to designate it as *sugary enhancer2*.

Conclusions and Future Research

A potential new starch metabolism mutant was discovered through EMS Mutagenesis of a *sugary1* line selected to mendelize the *sugary enhancer1* trait for genetic mapping. The location and nature of this new mutation is unknown, as is the biochemical basis for the phenotype that it produces. Further research is needed to elucidate its role and study its effects on the development of maize endosperm.

The most parsimonious hypothesis for the mode of inheritance of the *stipple* mutation of those analyzed above is that it is a recessive mutation in a paternally-imprinted gene. However, linkage was not considered in the above analysis, as the strength of linkage could skew the

genotypic and phenotypic ratios to a variable degree. Linkage could modify several of the ratios of the above hypotheses so that they could become more or less compatible with the nearly 1:1 segregation ratio observed.

The most immediate step would be to attempt to breed plants that are homozygous for the new mutation that do not also contain *se1* alleles, to determine the seed phenotypes more precisely. Subsequently, its biochemical impact on developing kernels and mature seeds should be assessed. Complementation tests with other known starch pathway mutants could determine if this mutation lies in a gene that has already been described or if it is indeed a new gene.

Mapping the location of this mutation in the current genetic background may prove to be challenging due to the highly isogenic nature of the *Se1* and *se1* lines used to make the initial hybrid. If *stipple* can be observed in a different genetic background through crossing, that may facilitate mapping. Otherwise, whole-genome sequencing and a comparison to the parent lines may reveal potential locations of the lesion, and a similar analysis of the transcriptome through RNA Seq may reveal genes with different levels of expression due to the new mutation. There are tools and techniques available to determine the nature of *stipple* which if confirmed, mapped, and cloned, can add to our knowledge of starch metabolism in maize, including potential applications in developing new varieties of sweet corn with unique characteristics.

REFERENCES

Neuffer, Coe, & Wessler, 1997. Mutants of Maize. Cold Spring Harbor Laboratory Press.

Williams WM, Satyanarayana KV, and Kermicle JL. 1984. R-Stippled maize as a transposable element system. *Genetics* 107:477-488.

APPENDIX B

POLLINATION METHODS VIDEO SCRIPTS

B.1 CORN POLLINATION

Introduction

Corn, or Maize, is one of the most important crops in the United States and around the world. There are currently many varieties of corn, such as field corn, popcorn, flour corn, sweet corn, and baby corn. Most of the corn grown commercially is a hybrid of two different inbred lines, from seeds that must be regenerated each year.

Since maize is an open-pollinated species, pollinations in a field will be random and unpredictable. Some varieties of corn are bred with Self-Incompatibility genes to prevent cross-pollination on the farm. This is particularly useful, for example, in growing popcorn to keep other maize varieties from reducing the popping quality of the kernels.

To breed new varieties with improved traits, or to maintain the integrity of popular cultivars, corn breeders have developed a simple system for making controlled crosses that can work at almost any scale, from a backyard garden to a large-scale breeding and seed production operation.

Basic Biology *(terms in* ***bold*** *displayed on screen)*

The scientific name for Maize is **Zea mays**, and it is **Diploid** with 10 pairs of chromosomes. (**20 Chromosomes**) It evolved through artificial selection from a wild grass in Central America called Teosinte. Teosinte still grows in the wild today, and maize will naturally outcross with this species.

Maize is **Diclinous** because it has separate male and female flowers.

Maize is also a **Monoecious** species because both male and female flowers are found on every plant.

The male flowers that produce the pollen are at the top of the plant in a structure called the tassel. The anthers start producing pollen grains as they emerge from the glumes and the pollen is dispersed by the wind.

The female flowers that produce the seeds can be seen on the sides of the plant, and are contained in a structure called the ear. These flowers collect the pollen with long, specialized stigmas called silks. Each silk leads to a single egg that grows into an individual maize kernel.

Equipment and Methods

Making controlled crosses can be made easy with a few pieces of simple equipment. Brown paper bags are used to collect pollen from the tassels, and small, white, glassine bags protect the silks from foreign pollen. The bags are treated to resist rain. You'll also need a permanent marker, a sturdy stapler, and a small, blunt knife. A utility apron will keep all of this with you out in the field.

The female flowers are the first to emerge, and their silks must be protected from foreign pollen. They first appear as shoots that grow out from behind some of the leaves. The first and uppermost shoot to emerge will usually produce the best ear. It is important to cover the young shoot before the silks emerge.

When you have found a sturdy shoot, tear off the blade of the leaf in front of it by pulling downward. The solid edge on the closed end of the bag can be pushed down behind the shoot to

make a slit in the lower part of the leaf. Open the bag and slip it over the shoot to keep pollen from falling on the silks when they emerge. The slit will hold the bag in place.

As the tassel matures, anthers gradually exert themselves and begin making pollen. A good tassel for collecting pollen has some anthers already emerged, but many still waiting to emerge and produce fresh pollen. You should also make sure that there are silks to pollinate before putting up your bags. If the silks take too long to emerge, you can speed up the process by cutting off the tip of the husk with your knife.

Pollen is shed mid-morning and in the afternoon, when morning dew has evaporated and the temperature is cool. The pollen only lasts for a few hours and is difficult to store, but this can work to the breeder's advantage: By putting a bag over the tassel, all of the pollen inside the bag, whether from that plant or from another, will be nonviable by the next morning. The only pollen that will be viable will be what the plant produces that morning.

First, the brown tassel bag must be clearly labeled with the genotypes of the parents, and the date. By convention, the genotype of the female flower receiving the pollen is put on top, and the male underneath. It is easy to remember if you think of the old adage, "Ladies First." Labeling the bag when you put it up helps you keep track of your crosses down the road, and the date can help you tell the difference between bags you just put up, and bags put up the previous day.

Place the bag over the tassel, fold in half at the bottom, and up from the bottom diagonally, which will keep the pollen from falling out. Staple the bag to keep it in place. If the stalk seems too weak to stand up to the wind, you can fold the bag around the uppermost leaf to give it stability.

The next morning, when pollen is being shed by nearby plants, tip the tassel bags to the side and hit them a couple of times to release the pollen from the anthers. Then, remove the staple and take the bag off without letting the pollen fall out of the bag.

To pollinate the silks, remove the white bag protecting them, and quickly dump the pollen on them. Place the labeled tassel bag over the ear, and staple the back two corners together on the other side of the stalk. Leaving two corners of the bag free will allow room for the growing ear.

Now you're done with your cross. When the ears have fully matured and set seed, they can be pulled off the plants, rolled up inside the labeled bags, and set somewhere to dry. Large operations often have seed drying facilities to make this process go faster.

Special Genetics Section: Hybrid Seed on a Commercial Scale

Hybrids of two inbred lines of maize are often larger and more productive, a concept known as **Heterosis** or Hybrid Vigor.

Once a desirable hybrid maize cultivar has been developed, the next step is to accomplish a controlled cross on a field-wide scale. To do this, several rows of the female parent alternate with a single row of the male parent. A specialized detasseling tractor is driven through the field, removing the tassels from the female rows, leaving only the "male" rows to produce pollen. So every ear of corn that grows on the "female" plants is a cross between the two parents.

Male-sterile varieties can be used to make controlled crosses, however, this method is not as extensively used in maize. It is widely used in other hybridizing crops such as rice and onions.

Conclusion

The seeds from the female rows of these large-scale crosses are collected and sold to farmers to plant the millions of acres of hybrid corn that are grown across the country, and around the world.

B.2 PEPPER POLLINATION

Introduction

Peppers are a diverse and important vegetable that can be found in cuisines around the world. While most peppers are valued for their spicy, pungent flavor, some can be sweet and even fruity.

Peppers can be green, yellow, orange, red and purple, and can be decorative as much as delicious. In sizes both large and small, they come in round, oval, tapered, curly, and sometimes bizarre shapes. The techniques that can be used to breed peppers with traits you desire are simple, and can be applied on both a small and large scale.

Basic Biology *(terms in* ***bold*** *displayed on screen)*

Peppers belong to the family **Solanaceae**, along with tomatoes, potatoes, and eggplants.

The pepper genus is called ***Capsicum***, which contains about twenty-seven species. They are all **Diploid**, and while some wild species have 13 pairs of chromosomes, most have 12 pairs of chromosomes, (**24 Chromosomes**) They originated in Central and South America in the tropics, and were domesticated many times. Although peppers are grown as annuals, most are

actually perennial plants, and some species can live as long as 30 years. Five species make up the common peppers that people grow and enjoy.

Capsicum annuum is the most important and commonly grown species, and usually has white flowers. It includes sweet, bell, and banana peppers, jalapeños, pimentos, cayenne and paprika.

Capsicum chinense, so named because it was originally thought to be from china, is actually from Central America. This species is where we find the spicy Habañero pepper, and the Jamaican Scotch Bonnet.

***Capsicum frutescens*,** which originated in Central and Northwestern South America, includes the Tabasco pepper and the Bird's Eye Chili, also known as Thai Hot peppers.

Capsicum baccatum, known as the Aji Pepper, is from Central South America. It has a distinct fruity flavor and can also be very hot. It also includes the Lemon Drop pepper, Piquante, and the oddly-shaped Bishop's Crown.

Capsicum pubescens is a hairy-leafed species from Central America and Peru. Although it is the earliest known domesticated pepper, it is less widespread than other peppers today. It includes the Rocoto, Chile Manzana, and Canario peppers.

Species in the Capsicum genus have a complex array of interrelationships, which determine which species can be crossed with each other. The cultivated Capsicum species are organized into three main groups called complexes (**Capsicum Complex**) to organize this information and help breeders determine what interspecific crosses are possible.

Capsicum annuum, chinense, and *frutescens*, and the wild species *Capsicum galapagoense* (***C. annuum, C chinense, C. frutescens, C. galapagoense***) are in the **Annuum Complex**.

Capsicum baccatum and the wild *Capsicum tovarii* and *praetermissum* (***C. baccatum, C. tovarii, C. praetermissum***) make up the **Baccatum Complex**.

Capsicum Pubescens and wild *Capsicum eximium* and *cardensii* (***C. pubescens, C. eximium, C. cardenasii***) together form the **Eximium Complex**.

There are other groupings of wild Capsicum species, but their relationships are less well understood. Some species, such as ***Capsicum chacoense***, are considered intermediate between different complexes.

Breeding within a species complex is usually simple, but between each complex is difficult. To move genes between species in different complexes, breeders can use rare **Bridge Species** such as ***Capsicum flexuosum*** and ***Capsicum parvifolium***.

Pepper species are **Monoclinous** because the flowers are bisexual or "**Perfect**," with both male and female organs.

Pepper flowers usually have five petals fused together at the base, and five green sepals which form the calyx. The **Stamens** which hold the pollen-producing anthers are **epipetalous**, meaning that they grow off of the petals. There are five stamens around the female pistil which contains the **ovary** and has a **Stigma** to receive the pollen. Although referred to as a vegetable, the pepper is technically a fruit, because the part that we eat develops from the ovary and contains seeds.

In the wild and on the farm, Peppers are cross-pollinated by insects, and the flowers can also self-pollinate. Growing your plants in a greenhouse can minimize accidental pollination and help you control your crosses.

Equipment and Methods

To make controlled crosses with peppers, you only need a few pieces of equipment. A pair of forceps will be necessary to perform the pollination, as well as tags and a permanent marker to label your crosses. A knife and a paper towel or newspaper is all that you will need to harvest your seeds. If you plan to store pollen in the long term, you will need small snap-cap tubes or gel caps and a container of desiccant. If you are working with hot peppers, gloves and goggles will protect you from the capsaicin during harvesting.

To maintain the integrity of your crosses, you should also clean your tools and hands with ethanol between each cross. Latex gloves can also help prevent cross-contamination. Pollinations are best done early in the morning when pollen is being produced.

To start your cross, first, you must select a young female flower that will bear a good fruit. Pepper flowers grow from the nodes of the stems where branches form. The first two tiers of flowers near the bottom of the plant will provide the best quality fruit with the most seeds. Select a flower that has not yet opened, and carefully tear open the petals with your forceps.

Next, emasculate by gently removing the anthers from the flower. By removing the anthers before they open, it will prevent self-pollination.

For the male parent, select a newly opened flower that is producing pollen. Remove it from the plant, and tear off the petals with your forceps. With your male flower, gently brush the stigma of the female flower to coat it with pollen. Pollen can also be scraped off and applied with your forceps, and can be saved in a snap-cap tube for the future. At zero degrees Celsius, pollen can last five to six days, but this can be extended to six months if they are kept dry with a dessicant.

Finally, label your cross with the parents, and the date. Remember to write the female parent first.

Harvesting seeds is very easy. When peppers are mature, they change color, and can be removed from the plants. With your knife, cut off the bottom of the fruit to open up the hollow seed chamber, and cut or tear the rest away. With your hands, gently rub the seeds off of the fruit and onto the paper towel to dry. If you are working with hot peppers, be sure to wear gloves.

When the seeds are dry, collect them in a labeled bag to plant the next generation.

This simple process can be done on a large scale to produce thousands of seeds. For hybrid seed production, breeders may use peppers that are male sterile, preventing the need to emasculate the female flowers. The male and female plants can be grown adjacent to each other in isolated fields to produce enough hybrid seeds for farmers to plant.

Special Genetics Section: Breeding Pungency

One of the most important traits of a pepper is its spiciness, or pungency. This 'hot' flavor is mainly caused by a molecule called **Capsaicin**, part of a group of related pungent compounds called **Capsaicinoids**.

Pungency is measured in Scoville Units, which refer to the degree it must be diluted for someone to not be able to taste the spice anymore. Today it is measured with more advanced techniques like High performance Liquid Chromatography, which measured the total capsaicinoid content.

Sweet peppers start at a Scoville rating of around zero, while mild pepperoncini's will be 100-500, Jalapeños are in the thousands, and hot peppers such as Bird's Eye chilis and Habaneros can have around 100,000 units. This trait can be changed through breeding. One of

the hottest peppers in the world, the **Naga jolokia** pepper which has a Scoville rating of up to 1 Million, is a hybrid between the Capsicum chinense and frutescens (**C. chinense x C. frutescens**) species.

Conversely, breeders have been able to achieve varieties of hot peppers such as jalapeños that have no pungency at all, for people who are sensitive to spice.

Conclusion

There is a lot of room in Capsicum species for new and interesting combinations, enough to delight any breeder, and eater, of peppers.

B.3 SOLANUM POLLINATION

Introduction

Tomatoes and Potatoes are closely related vegetables, and are popular throughout the world. Tomato fruits can be small and cherry-like, or large and beefy, from red, to yellow and orange, and some even have a slight purplish color. Potato tubers can be round, oblong, smooth, lumpy or scaly, from white and golden in color, to red and blue.

Although we completely different parts of these two plants, they are closely related and share many characteristics in common, so new varieties of these species can be made with the same simple techniques.

Basic Biology *(terms in* ***bold*** *displayed on screen)*

Tomatoes and potatoes belong to the nightshade family **Solanaceae**, along with peppers and eggplants.

The scientific name for the tomato is ***Solanum lycopersicum***, and it is diploid with 12 pairs of chromosomes. (**24 Chromosomes**) It was domesticated in the Andes Mountains of South America, and can cross with many wild species.

The cultivated tomato fruit is edible, but wild species of tomato can have poisonous fruits, which breeders need to keep in mind when crossing with wild relatives. The genes that cause this can be eliminated by crossing repeatedly with the cultivated tomato, a process called **Back Crossing**.

The potato belongs to the species ***Solanum tuberosum***. The most widely cultivated potato is **Tetraploid** with four copies of each of 12 chromosomes, (**48 Chromosomes**). It originated in South America, cultivated by the Inca as long as 10,000 years ago. Its wild relatives live throughout the Americas and are mostly **Diploid** with 12 pairs of chromosomes, (**24 Chromosomes**), however, other ploidy levels exist. The wild relatives of potatoes are numerous and their compatibilities are complex.

Solanum species are **Monoclinous** because the flowers are bisexual or "**Perfect**," with both male and female organs. They are therefore also **Monoecious** because every plant has male and female parts.

The flowers of tomatoes and potatoes are very similar to each other. They typically have five green sepals behind five petals that are partially fused together. In the potato, the stamens are separate and hold five pollen-producing **Anthers** around the female pistil which contains the **ovary** and has a **Stigma** to receive the pollen. In the tomato, the anthers are fused together in a cone (**Anther Cone**), which can make controlled pollinations a little more challenging.

The flowers of both species are capable of self-pollination and cross-pollination by insects, so care must be taken to prevent undesired pollinations from occurring. Growing them in a greenhouse is the best way to prevent this from occurring.

Equipment and Methods

Making controlled crosses of tomatoes and potatoes efficiently requires several pieces of equipment. The most important is a device to dislodge the pollen from the anthers. Some companies make specialized devices, but you can modify the tip of a vibrating toothbrush with a piece of flexible rubber, which will do the trick. To store the pollen between crosses or over the long term, you will need gel caps, or small snap-cap tubes if the air in your greenhouse is too moist for the caps. To keep the pollen dry, use a container of desiccant, such as non-clumping cat litter. Fine-tipped forceps are optional for potatoes but a must for crossing tomatoes, and fine gauze or cheesecloth will be necessary for both. Tags and a permanent marker will help you keep track of your crosses, and a knife, scooping tool or spoon, and strainer will aid in tomato seed harvesting. Finally, you will need some small containers for seed harvesting.

To maintain the integrity of your crosses, you should also clean your tools and hands with ethanol between crosses. Latex gloves can also help prevent cross-contamination.

Pollinations are best done in the morning when pollen is being produced.

Potato:

Potato flowers are the easier of the two to cross. Begin by selecting a mature pollen-producing flower, fold the petals back, and place the anthers just inside your collection tube.

Turn on the vibrating toothbrush and gently touch the flower. The pollen will come out of the front of the anthers and into your tube. Eight to ten good flowers will give you enough pollen for over a hundred crosses.

Store the tube of pollen in your container of desiccant to keep it dry and viable between crosses. Potato pollen can last several months to a year or more if stored dry in a freezer at minus 20 degrees Celsius.

Potato flowers in each inflorescence mature in pairs every few days. Remove any mature flowers to keep them from pollinating your cross. To select a flower to pollinate, find a pair of unopened flowers that have petals that are beginning to change color. Younger flowers can be left on if you plan to use them to make crosses, otherwise remove them.

Carefully remove the petals with your hands or forceps, exposing the interior of the flower. The flower must be emasculated to prevent self-pollination. Carefully remove the immature anthers without damaging the stigma.

To pollinate your flower, insert it into the tube of pollen, gently brushing the side of the tube with the stigma.

Your cross is now finished. Label a tag with the date, and the two parents of your cross. By convention, the female parent goes first. Tie the label to the stem beneath your cross.

A few days later, if you see a fruit developing on your flower, your cross was successful. Wrap the fruit with a small square of cheesecloth in case the fruit falls off before you collect it. You can use the string from the tag to tie the cheesecloth on, or you can use a twist-tie if you have one.

Three weeks after pollination, remove the potato fruits from the plant, and set them aside for another three weeks for the seeds to finish developing.

To extract the seeds, squeeze the fruit into a tub of water, rolling it around in your fingers. The viable seeds will sink to the bottom, so pour off any that float. To collect the tiny potato seeds, filter the water through the cheesecloth and set them somewhere to dry.

Tomato:

Tomato flowers are smaller and more delicate than potatoes, and can be a bit more challenging to cross. Magnifying goggles can help you see the finer details, but they are not necessary.

To collect pollen, place a mature flower inside your collection tube and vibrate the pollen out of the anthers as before.

Pollen can also be collected on a mass scale by collecting mature anthers in a tube or Petri dish. Place the collected flowers in the sun or at least 18 inches below an incandescent lamp for 24 hours to dry. The pollen can be gathered by sifting the contents with a fine screen.

As long as it is kept dry, tomato pollen can be stored for long periods of time in a freezer as with potatoes.

Tomato flowers mature from the bottom of the inflorescence, with the youngest flowers toward the tip. They open about one per day, and successful crosses are made with flowers that are two days before opening. Remove any flowers that have already opened, and select an unopened flower that does not have any color in the petals.

Some breeders remove one or more sepals to indicate the flowers that they have crossed. Using your forceps, carefully remove the petals one by one. The immature anther tube can now be seen, which must be gently teased off of the flower. Sometimes you will need to sever the

cone at its base with your forceps, but it can usually be pulled off the flower. If the pistil is damaged you will need to try again with a new flower.

To pollinate your flower, gently brush the inside of your pollen collection tube with the stigma. To ensure that the stigma is receptive, you can also apply pollen a second time the following day. Once you have a successful cross, label it with the parents and the date, and wrap the developing fruit with cheesecloth.

When your fruit has fully matured, cut them open and scoop out the seeds into an open container. Tomato seeds are held in a gelatinous matrix, which can be removed by fermentation. Leave them at room temperature for 1-2 days; a mold may form. When the seeds are free from the gel, rinse them with water in the strainer, and set them out to dry. Finally, transfer your seeds to labeled bags to plant the next generation.

When a desirable tomato variety has been developed, seed producers can increase the seed on a mass scale, or for hybrids, conduct multiple replicated crosses.

Potatoes, on the other hand, are vegetatively propagated by planting the tubers, or seed potatoes. The resulting plants are genetically identical to the original. But in order to go from one plant to acres of farmers' fields, a sample of tissue is used to culture thousands of plants in a lab. These are then planted to grow the many varieties of potatoes that people enjoy.

Special Genetics Section: Potato Ploidy

Although cultivated potato varieties are diverse, wild relatives represent a valuable resource for useful traits. For example, *Solanum bulbocastanum* (***S. bulbocastanum***) is the source of late blight resistance, and many processing potatoes have *Solanum chacoense* (***S. chacoense***) in their pedigree.

There are about one hundred and twenty known species of potato, and about three quarters of them are diploid, and some can be **Triploid**, **Tetraploid**, **Pentaploid**, and even **Hexaploid**. In order to have a fertile cross, the species need to be at the same ploidy level, with the same number of chromosome copies. Breeders have a few special techniques to navigate up and down ploidy levels.

To go from tetraploid to diploid plants, breeders have to reduce the number of chromosome copies by half. To do this, they can use one of several diploid "**Phureja**" species to pollinate the tetraploid. The sperm cells in the Phureja Group will not fertilize the egg, but will allow the embryo to develop. The resulting "Haploid" seed will have two copies of each chromosome, half the number of its parent. When grown, these can cross with other diploids.

To move in the other direction, up the ploidy scale, breeders have two techniques. First, plants can be treated with a compound called Colchicine, which doubles the number of chromosomes. Alternately, a few pollen grains from diploid plants may naturally have twice the number of chromosomes. This 2n gamete is compatible with tetraploid plants, allowing them to be crossed.

Finally, not all potato relatives can cross with each other, even though they have the same number of chromosomes. Potato species are divided into several groups assigned a number, called the **Endosperm Balance Number**, or EBN. Species must have the same EBN number to be compatible. For example, diploid with an EBN of 1 cannot cross with another diploid with an EBN of 2.

Recently, however, potato breeders have discovered one species, ***Solanum verrucosum***, which can cross with both 1 EBN and 2 EBN species. This **Bridge Species** may allow valuable new traits to be introduced into potato varieties.

Conclusion

Whether breeding within cultivated tomato and potato species, or crossing with their wild relatives, the diversity of Solanum is enough to keep any plant breeder busy.

B.4 CUCURBIT POLLINATION

Introduction

Cucurbits are a very diverse group of crops. From squash, to melons, cucumbers, and gourds, about a dozen of eight hundred species in this family of plant have been domesticated. The productive vines of these plants grow along the ground and many can be trained to climb trellises, which appeals to gardeners. The wide variety in shapes, colors, textures and flavors of cucurbit fruits provides many opportunities for creative combinations. These can be achieved in a few easy steps.

Basic Biology (terms in **bold** displayed on screen)

Cucurbits belong to the family **Cucurbitaceae**, and although they are closely related only some domesticated species can interbreed. The scientific name for the cucumber is ***Cucumis sativus***, and it is diploid with 7 pairs of chromosomes. (**14 Chromosomes**) It originated in India, and can cross with its wild relative, *Cucumis sativus var. hardwickii* (***C. Sativus var. hardwickii***).

Cucumis melo, the true melon, includes fruits such as the muskmelon, casaba, cantaloupe and honeydew. It is diploid with 12 pairs of chromosomes, (**24 Chromosomes**) although some varieties are **Tetraploid** with four copies of each chromosome. (**48 chromosomes**) It originated in Persia, and breeders are working on finding compatible wild relatives.

Citrullus lanatus, the Watermelon, is diploid with 11 pairs of chromosomes (**22 Chromosomes**). This originated in Africa, and can still cross with its wild relative, ***Citrullus colocynthis***, also known as the Bitter Apple or Desert Melon.

There are four species of cultivated cucurbits that are interfertile, owing to the fact that they are all diploid with 20 pairs of chromosomes (**40 Chromosomes**). They originated in the Americas about 8 to 10,000 years ago, and bear so many similarities that they are often collectively called the squash or pumpkins.

Cucurbita pepo is the summer squash, and includes the zucchini crookneck, acorn and spaghetti squash. The wild relatives include ***Cucurbita pepo* var. texana**, the Texas Gourd, and ***C. fraterna***. ***Cucurbita maxima*** is the winter squash, and includes the buttercup, hubbard, banana squash, and the giant pumpkins. It evolved from a wild species called **C. maxima subsp. andreana.** ***Cucurbita moschata*** includes butternut squash, the calabaza, and some pumpkins, but its wild ancestor remains unknown. ***Cucurbita argyrosperma*** is the Cushaw pumpkin, and evolved from ***C. argyrosperma* subsp. Sororia.** Another wild relative that it can cross with is ***C. argyrosperma var. palmieri***.

Luffa aegyptiaca, the Luffa Gourd, is Diploid with 13 pairs of chromosomes. (**26 Chromosomes**) This gourd, valued for its spongy seed matrix, originated in India.

Despite the variation between these species, their reproductive systems are essentially the same. Cucurbits are **Diclinous** because they have separate male and female flowers.

Cucurbits are also **Monoecious** because both male and female flowers are found on each plant. Some varieties of cucumber may have only female flowers, so they are called **Gynoecious**. Some cucurbit varieties, especially melons, can also be **Andromonoecious**, with both male flowers and bisexual flowers that have male and female parts.

The two flower types are easy to identify. Female flowers have an organ called the Ovary at their base, which develops into the fruit after pollination. Inside the Female flower is an ornate Stigma that receives the pollen. The male flowers do not have an ovary, and have up to five anthers fused together, which produce the pollen.

In some cucurbit species, the male flowers may present themselves on long pedicels. Since the male flowers are the first to emerge, they attract bees and other pollinating insects which become coated with pollen to be transferred to the female flowers.

Because insects are difficult to control, cucurbit breeders often bring their plants inside into a greenhouse, where controlled pollinations can be made. Cucurbits may have many branches, so removing a few as the plant grows can help keep your greenhouse from becoming overgrown.

Equipment and Methods

Cucurbits are among the easiest of crosses to make. You will need twist ties to make crosses, a knife to cut open the fruits, and a strainer with a few small containers to collect your seeds. Labeled tags and a permanent marker can help you keep track of your crosses down the road.

Cucurbit crosses are best done in the morning, when the plants are the most receptive to being pollinated.

Begin by selecting a new male flower that is producing pollen, and remove it from the plant. Carefully remove the petals without touching the anthers.

Then select a newly opened female flower. Place the male flower inside of the female flower, just touching the anthers and stigma together. With your other hand, bring a twist tie over

the petals and gently clamp it down. Folding the tie over itself at the end will hold it in place. Some breeders will use a paint brush to transfer pollen from one plant to many flowers, but it is difficult to clean between crosses.

Now that you are finished with your cross, you should label it. Some breeders use color-coded twist ties, but the most common and versatile way to keep track of your cross is with a marking tag. First write the female parent, and then the male parent. Don't forget to write the date. Gently tie it below the flower.

As the fruits grow, it is also a good idea to label them with a permanent marker in case they fall off of the plant. When the fruits are fully ripe, in this case yellow, remove them from the vine and set them aside for a week so the seeds can mature.

When you are ready to collect the seeds from your fruits, cut them open with a knife and scrape the seeds into the strainer. Take care to cut only through the outer flesh of the fruit to avoid damaging your seeds. Rinse them with water, and place them in an open container to dry.

Some cucurbit species may require fermenting the seeds for a day to release them from the stringy flesh that holds them inside the fruit. When the seeds are dry, put them in labeled bags to be planted the next year.

On a larger scale, controlled crosses for hybrid seed production can be made out in the field. Seed producers will plant two parental lines in isolation, and bring in hives of bees to pollinate them. To ensure that every seed comes from a cross between the two parents, breeders remove the male flowers from the female parent before they open. Alternately, they can use gynoecious plants as female parents, or treat the female parent with a hormone that suppresses male flowers and increases the number of female flowers. Either of these methods can save time and ensure that the seeds are hybrids of the two parents.

Also, in order to self-pollinate and maintain gynoecious varieties, breeders can artificially induce male flowers by treating the plants with another compound. These methods give seed producers many robust options for making hybrid seeds.

Special Genetics Section: Seedless Watermelons

Particularly noteworthy is the seedless watermelon, which was first developed in the 1950s and is very popular today. To develop a seedless watermelon variety, a **diploid** seedling is treated with a compound called **colchicine**. This doubles the number of chromosomes in the plant, turning it into a **tetraploid**. This is then used as a female parent to cross with another diploid, producing **triploid** seeds with three copies of each chromosome.

These triploids can develop and produce fruit, but the odd number of each chromosome causes problems when the cells undergo meiosis to produce eggs and pollen. As a result, the seeds in the fruits do not develop. In order to grow seedless watermelons, farmers plant one fourth of their fields with a normal diploid variety known as a **pollenizer** to pollinate the sterile triploids.

Although watermelon breeders have to evaluate two parental lines after they have been combined as a triploid, and farmers have to leave space in their fields for a pollenizer, the extra effort it takes to make seedless watermelons is worth it.

Conclusion

These various techniques are used by breeders throughout the world to create the many acres of diverse cucurbits that we all enjoy.

B.5 CARROT AND BEET POLLINATION

Introduction

Carrots and Beets are important root vegetables eaten in many places around the world. Whether eaten fresh, boiled, or pickled, these sweet vegetables are a delightful addition to a variety of meals. Carrots can have several different colors from the common orange to red, yellow, purple, and white. Beets include the familiar table beet, chard, spinach beets, and sugar beets grown for producing table sugar. Root beets range from white and yellow to a very deep red, and they can also have stripes. Beet greens are also edible, and like chard, can have colorful midribs.

The techniques used to make controlled crosses of carrots and beets are simple, and can be expanded from a back yard to a commercial breeding program.

Basic Biology *(terms in* ***bold*** *displayed on screen)*

Carrots belong to the family **Apiaceae**, along with parsnips, celery and many spices. The scientific name for the carrot is ***Daucus carota***, and it is diploid with nine pairs of chromosomes. (**18 chromosomes**) The ancestor of the carrot is considered to be the same species, and is from the Middle East, Europe and Africa. Around the world, today it can be seen growing on the side of the road or in gardens, and is also known as Queen Anne's Lace. Two other sexually compatible wild species are *Daucus capillifolius* and *Daucus sahariensis*. (***D. capillifolius, D. sahariensis***) The carrot is a recent domestication, and has only been eaten for the last thousand years or so.

Beets belong to the family **Chenopodiaceae**, along with spinach and amaranth. The scientific name for the beet is ***Beta vulgaris***, and it is also diploid with nine pairs of chromosomes. (**18 chromosomes**)

The wild ancestor, ***Beta vulgaris ssp maritima***, also known as the Sea Beet, grows in the Mediterranean, Europe and some parts of Southeast Asia. The beet was domesticated at about 4,000 years ago.

Carrots and beets are both **Monoclinous** because they have **Perfect**, bisexual flowers with both male and female parts. Carrots have many tiny flowers that grow in an umbrella-like arrangement called an Umbel. The individual flowers have five petals and five stamens that have the pollen producing **Anthers**. There are two female **Stigmas** that each lead to an ovary that can together produce a total of two spiny seeds. Male sterile carrots actually produce a second row of petals instead of stamens

Beets also have very small flowers, but they are arranged in a vertical inflorescence called a panicle. Each flower has five green to reddish tepals, rather than distinct petals and sepals. The flowers have five stamens and three stigmas, and produce an aggregate fruit that is represented by a single "seed ball." This seed ball can contain multiple embryos, but typically no more than six or seven.

While the flowers of these species can be emasculated and crossed like other bisexual flowers, their small size makes this impractical. Instead, breeders can take advantage of the natural cross-pollination mechanisms of these two crops to get the job done.

While carrots are pollinated by insects, and beets are primarily wind-pollinated, the life cycles of these two plants are so similar that they can be grown side by side.

Both carrots and beets are biennial (**Biennial**) crops, which means that they normally take two years to flower. To get these two plants to flower in the same year that they are planted from seed, they must be "vernalized" by simulating the conditions of winter. After growing plants to maturity in the field or greenhouse, the roots should be stored in a refrigerator for six to eight weeks, at about 3 degrees Celsius. A standard kitchen fridge will work. It may be necessary to pack the taproots in a paper bag full of wood shavings to help prevent rotting.

When the roots have been vernalized, this is a good time to check their quality before planting. Slicing the bottom off of carrots diagonally will not only indicate the health of the plant, it will also make them easier to plant into pots in a greenhouse. Beets can also be sliced, and a lengthwise cut will tell a lot about the condition of the root, and near the top will allow you to examine the rings. The plants will grow despite being damaged.

If you follow this procedure, seeds planted in a summer field can produce flowering carrots and beets in a winter greenhouse, and vice versa.

Equipment and Methods

The equipment necessary for crossing carrots can seem a little complex and strange, but it works well. Since insects pollinate these flowers very well on their own, breeders put netted enclosures around the plants and introduce flies to pollinate them. Common houseflies will work, but commercially raised blue bottle flies are often used.

Large cages can hold several plants, but if all you need to cross are two plants in pots, you can set up a small netted enclosure that will do the job. First, build a frame out of sturdy wire, with a loop on the top and bottom. Fashion a cloth bag that will fit around the wire frame, with enough material to cinch it at the top and bottom. To use it in a cross, hold up the frame

with a wire hung from above, or with stakes set in the pots, and gather the two plants together inside the frame. Drape the cloth over the plants, and tie it together at the bottom with a twist tie. At the top, a tube with a cork can provide an easy way to introduce flies into the bag.

When the flowers are ready to be pollinated, introduce the flies or their pupae, and close it up again. The flies will be attracted to the flowers and move the pollen around for you. You may need to add flies each week to ensure that all of the flowers are pollinated. Be careful not to let flies out of the bag or they might bring pollen to your other plants. Now label your cross with the parents, and the date. Remember to write the female parent first.

Crossing beets is straight-forward. The equipment you will need is a long paper bag to go around the plants, a tag and a pen or pencil to label your cross, and a stapler to hold things together. A piece of cotton will keep your seeds from falling out of the bag.

Select two beet plants that you want to cross, and put a stake in one of the pots to hold up the bag. Bring the stems together, and wrap the stems with the piece of cotton. Remove the secondary stems and flowers. Place the paper bag over the top of the plants, and fold it tightly around the cotton. Fold the bag up diagonally from the bottom and staple the bag so that no pollen or seeds can come out. Staple the bag to the stake, and your labeled tag to the top of the bag. Finally, make sure the bag is inflated to pollen has room to move around.

Since beet flowers open daily and the anthers fall off before noon, you will need to visit your plants to lightly shake or flick the bags to release the pollen so that fertilization can occur. Beets can also be pollinated in a block, such as for maintaining a diverse population. This should be isolated from other plants to maintain the genetic identity of the plants.

Male sterile plants are used in both carrot and beet breeding to ensure that the pollen only came from one of the two plants, although very often, the first crosses will be between two fertile

plants. In order to determine which seeds came from a self-pollination and which came from a cross, you will need to examine the progeny when they are grown out. A simple trait that is different between the two parents such as color can help you determine if your cross was successful. When the plants are very similar this becomes difficult, so today breeders use tools such as molecular markers that can test the DNA of each plant to make this determination.

Harvesting carrot seeds is easy. After two months, the seeds will be fully mature and the umbels will be dry. Carefully remove the umbels and place them in a labeled bag for processing.

For beets, cut the plants off at the base and set the bags on their sides to dry. When these are dry, carefully open the bottom of the bag so that the seeds do not fall out everywhere. A tray can help you collect them for putting in a labeled bag.

Carrot and beet seeds require some extra effort to prepare for planting. Carrots have two spiky seeds attached to one another, which must be removed from the umbel and separated from one another. Beet seed pods are also rough and hard. You can smooth out and separate the seeds by rolling them under a piece of hard ribbed rubber, and a sieve can be used to let finished beet seed balls fall through. Carrot seeds can also be processed with your hands.

To separate the seed pods from the chaff, gently blow them in a dust pan while shaking. Larger operations separate seeds with a mechanical blower. Heavy, high-quality seed pods remain near the bottom, while chaff and low-quality pods blow out the top. The seeds are now ready to plant.

Special Genetics Section: Plant Pigments

The colors of carrots and beets are not only attractive, but they also indicate the healthful properties of these vegetables. Carrot colors are caused by a class of pigments called

Carotenoids, many of which are precursors for Vitamin A. The orange color of the common carrot today is caused by Beta Carotene, and orange carrots have only been around for the last 300 years. In the last few decades, carrot breeders have even enhanced the amount of beta-carotene in carrots, making them an important source of this pro-vitamin.

In beets, the yellow and red colors are caused by pigments called **Betalains**. These are antioxidants, and some beets have been bred to have very high levels of these betalains. The red pigment is now also used as a food coloring.

Conclusion

Whether you are looking for a healthy addition to a meal, or to create a living work of art, breeding carrots and beets can be easy and rewarding.

B.6 FRUIT TREE POLLINATION

Introduction

Tree fruits from peaches to apricots, oranges and lemons, and apples and pears are a delicious and nutritious part of every day life, especially in the summer.

There are many different types of fruit trees and vines, each with their own unique characteristics. Here, we will focus on three major groups: The pome fruits, stone fruits, and citrus. With apple crossing techniques as an example, you should be able to cross many different types of fruit and nut trees.

Basic Biology *(terms in* ***bold*** *displayed on screen)*

Apples, pears, and quinces are in the rose family, **Rosaceae**, and are all typically **Diploid** with 17 pairs of chromosomes. (**34 Chromosomes**). Some apple varieties can also be triploid or tetraploid. Triploid varieties are very juicy because the polyploid cells are larger, a concept called **Gigas**.

Malus domestica, the apple, descended from the wild species ***Malus sieversii*** in central Asia. There are over 50 wild Malus species that are found in Europe and Asia, some of which are sexually compatible with the domesticated apple.

There are more than 30 different species of pears in the ***Pyrus*** genus, which grow throughout Europe and Asia. The three main cultivated species are the European pear, (***Pyrus communis subsp. communis***), the Chinese white pear, (***Pyrus x bretschneideri)***, and the Nashi pear. (***Pyrus pyrifolia***.)

The quince ***(Cydonia oblonga)*** is from the mountains of central Asia.

Stone fruits are in the genus, ***Prunus***, which is also in the Rose family. They are mostly **Diploid** with 8 pairs of chromosomes, (**16 Chromosomes**), but some can be tetraploid or hexaploid. Prunus species are found in Asia, Europe, the Middle East, and even the Americas.

They include Peaches and Nectarines, Apricots, Plums, Cherries, and Almonds, which are eaten as seeds. Many stone fruit species with the same number of chromosomes can cross with each other. The pluot and plumcot are examples a cross between plums and apricots.

Finally, the ***Citrus*** genus is a diverse and fragrant group of fruit tree species. They are **Diploid** with nine pairs of chromosomes (**18 chromocomes**), and some seedless varieties such as limes are also triploid. Some citrus fruit varieties can also develop seedless fruits even if they are not pollinated, a trait called **parthenocarpy**.

Citrus species are found throughout Southeast Asia and India, and even Australia. They include the Mandarin Orange, Pomelo, Citron, and Key Lime.

Citrus species are interfertile, and many important varieties are crosses between two or more species. The sweet orange (***Citrus x sinensis)*** is believed to be a cross between the mandarin and pomelo, and the lemon, a cross between the pomelo and citron. The grapefruit (***Citrus x paradise)*** is a further cross between the sweet orange and the pomelo. But the origins of the Tahiti or Persian Lime (***Citrus x latifolia)*** remain unknown.

Pome fruits, stone fruits, and citrus are **Monoclinous** because they have **Perfect**, bisexual flowers with both male and female parts. Like other members of the rose family, the pomes and stone fruits have five petals and five sepals, which surround many **anthers** and a single **stigma** that leads to the **ovary**. Pomes may produce up to five seeds, but each stone fruit contains only one seed. These species flower in the early spring. Citrus flowers, which may emerge at different times of the year depending on location and climate, typically have five petals and sepals, but can sometimes have only four. Citrus also have many anthers and a single stigma, however the fruits can contain many seeds. Citrus seeds can have multiple embryos, and can also even be clones of the maternal parent, a concept called **apomixis**.

Equipment and Methods

Making crosses between apple trees is a straight-forward process with a few important things to remember. When the flowers are mature and open, they produce pollen and can be pollinated by bees. To prevent this from occurring, simply cover the branch of the tree with a bag before the flowers open.

Many fruit tree species are self-incompatible, which can be advantageous because it can make the crossing process go quicker. Apples are almost exclusively cross-pollinated, however, in the right climate such as the Northwest coast of the United States, some pollen tubes can grow from a self-pollination and fertilize the egg. Keep this in mind before setting up your cross.

To make a cross with a self-incompatible apple, collect pollen from several flowers of the male parent with a pair of forceps. Collecting the anthers will get you lots of pollen.

Next, remove the bag protecting the flowers on the female parent, and brush the pollen onto the stigmas of each flower. You can use a paintbrush or forceps, but some breeders use their fingertips. Because pollen from these flowers can contaminate your container of pollen, you will need to clean up with ethanol before you go back to the container. If you pollinate with your fingertips, you can do ten pollinations before you need to wash your hands.

If your trees are self-fertile you do not need to bag the branch before the flowers open, because you will dissect flowers while they are still closed. As apple flowers mature and before they open, they pass through a stage called the "**Balloon Stage**". Flowers at this stage are fertile, but do not shed pollen. To make a cross, carefully tear off the petals with your forceps, and then remove the immature anthers. Take care to remove all of them. Now, simply pollinate the stigma as before.

Also remove any flowers that have already opened, and if you are done pollinating, remove any remaining buds, and label the branch that your pollinations were on. Remember to write the female parent first. To protect your pollinations from visiting bees, you can put a bag back over the branch.

Since apples flower once per year, there are a few tricks to make successful crosses between varieties. To speed up flowering, you can remove a few branches from a tree and put

them in a jar of water indoors. The flowers will quickly bloom and produce pollen. You can also collect and store tubes of pollen in a refrigerator for weeks, or if desiccated and dry, for months in a freezer. This enables breeders to even make crosses between trees in different places around the world.

When the fruit is fully ripe, remove it from the tree. To collect the seeds without damaging them, slice it open near – but not though – the center. Remove the seeds with a small spatula, rinse and allow them to dry, and place them in a labeled bag to plant the next generation. These can be planted in flats and transplanted to the field to grow into trees.

Special Genetics Section: Tree Breeding

Fruit trees can take several years to flower, and even longer to reach full maturity to evaluate. Because seeds from each tree will all be different from one another, when a tree with desirable characteristics has been found, it is vegetatively propagated to maintain the variety. Cuttings of branches and buds called **Scion**s can be grafted onto **rootstock**s to generate many more trees for full-scale evaluations, or to plant orchards for production. Because of this grafting process, breeders often select good rootstock and scion genotypes separately, and then graft them together for the best combination.

Conclusion

Fruit tree breeding is a patient process, as it can take twenty to thirty years to go from an initial cross to a new variety that you can find in the store. But with the diversity of shapes, colors, flavors, and textures, and the nutritional benefits that come with each new variety, the fruits of this labor are well worth it.

APPENDIX C

M4V FILES OF PLANT BREEDING EDUCATIONAL VIDEOS

Table C.1. *Fields of Study* and *Pollination Methods* video files

Title	Filename	Size (MB)
Fields of Study - Corn Breeding	FieldsofStudy-CornBreeding.m4v	31.6
Fields of Study - Apple Breeding	FieldsofStudy-AppleBreeding.m4v	46.9
Fields of Study - International Breeding	FieldsofStudy-InternationalBreeding.m4v	42.4
Fields of Study - Pepper Breeding	FieldsofStudy-PepperBreeding.m4v	21.2
Fields of Study - Switchgrass Breeding	FieldsofStudy-SwitchgrassBreeding.m4v	41.0
Fields of Study - Watermelon Breeding	FieldsofStudy-WatermelonBreeding.m4v	38.9
Pollination Methods – Carrots and Beets	PollinationMethods-CarrotsandBeets.m4v	83.3
Pollination Methods - Corn	PollinationMethods-Corn.m4v	54.2
Pollination Methods - Cucurbits	PollinationMethods-Cucurbits.m4v	74.2
Pollination Methods - Fruit Trees	PollinationMethods-FruitTrees.m4v	69.8
Pollination Methods - Peppers	PollinationMethods-Peppers.m4v	74.1
Pollination Methods - Solanum	PollinationMethods-Solanum.m4v	99.7