

The Genetic, Developmental, and Molecular Bases of Fruit Size and Shape Variation in Tomato

Steven D. Tanksley¹

Departments of Plant Breeding and Plant Biology, Cornell University, Ithaca, New York 14853

INTRODUCTION

Fruit, corresponding to the plant's ovary (or ovaries), protect seed development and serve as the vehicle for seed dispersal to different habitats for species propagation. Fruit also provide humans with a source of nutrition, culinary diversity, and often great pleasure. Humans consume fruit from a wide range of plants, including members of the Rutaceae (citrus), Rosaceae (stone fruits, apples, pears), Solanaceae (eggplant, pepper, tomato, husk tomato), Cucurbitaceae (melons, squash, cucumbers), Ribaceae (berries), Vitaceae (grapes), and a multitude of additional species.

Although fruit-bearing crop species are taxonomically diverse, they share a common feature. Fruit from domesticated species often have been tremendously enlarged over that normally found in the progenitor wild species. For example, the putative wild ancestor of the cultivated tomato, *Lycopersicon esculentum* cv *Cerasiforme*, bears fruit (composed of two locules) weighing only a few grams. By contrast, a single fruit from a modern tomato variety may contain many locules and weigh up to 1 kg, a nearly 1000-fold increase in weight (Figure 1A). A similar situation exists for most other domesticated fruit species (Smartt and Simmonds, 1995). In addition to increases in fruit size, the domestication of fruit-bearing species often has resulted in tremendous shape variation: wild and semiwild forms of tomato bear fruit that are almost invariably round, whereas cultivated tomatoes come in a wide variety of shapes: round, oblate, pear-shaped, torpedo-shaped, and bell pepper-shaped (Figure 1).

The topic of fruit growth and development can be approached with several questions in mind. From a global perspective, one can ask, What are the genes, proteins, and processes that specify or affect the formation of fruit? Of course, the answer to this question is that many if not most plant genes/proteins/processes influence fruit development. Plants compromised in photosynthesis, phloem transport, floral initiation/development, or male or female fertility either cannot produce fruit or are abnormal in their fruit production (e.g., parthenocarpic fruit, reduced fruit size, or reduced fruit load). As a result, many if not most tomato mutants have some alteration in their fruit phenotypes (Balbi and Lomax, 2003).

Another question regarding fruit growth and development is, What are the underlying genetic, molecular, and developmental changes that permitted wild progenitors to produce the large,

highly variable edible fruit associated with modern agriculture? Because this question is more narrowly defined, it is more likely to yield specific answers concerning processes unique to fruit development and relevant to agriculture. Thus, this review is focused on the question of how tomato fruit were changed by domestication. In approaching this topic, I try to summarize what is known about the alteration of fruit development during tomato domestication and to set forth a series of questions that need to be answered before we can claim any general understanding of this topic.

HISTORY OF TOMATO FRUIT DOMESTICATION

L. esculentum was domesticated by native Americans, possibly in Mexico, and by the time Europeans arrived, large fruited types already were in existence (Jenkins, 1948; Rick, 1995). Because domestication occurred in prehistoric times, no one knows the actual evolutionary pathway by which wild species gave rise to plants with larger and variably shaped fruit. The most likely scenario is that early humans selected for mutations associated with larger fruit, and gradually, enough "large-fruited" mutations accumulated to give rise to our present-day cultivars. Genetic analysis of crosses between cultivated species and their wild relatives supports this idea, because progeny from these crosses almost always segregate in a continuous manner with respect to fruit size, indicating that the domestication process involved mutations at a number of different genetic loci (MacArthur and Butler, 1938; Banerjee and Kalloo, 1989).

The quantitative nature of fruit size variation has severely inhibited the use of classic Mendelian techniques to identify and characterize the individual gene mutations associated with fruit domestication. Only recently has significant progress been made in identifying and cloning the underlying genes. The goal of this review is to both summarize our current knowledge of this topic and to frame some of the unanswered questions concerning the developmental and genetic control of fruit size and shape. However, before launching into a detailed discussion of this topic, it is worthwhile to summarize what is known about tomato fruit development.

QUALITATIVE DOMAINS OF TOMATO FRUIT DEVELOPMENT

A tomato fruit is classified as a fleshy berry and is composed of an epidermis, a thick pericarp, and placental tissues surrounding the seeds. The pericarp is the outer wall of the gynoecium, which is composed of at least two carpels (this number can be much

¹ To whom correspondence should be addressed. E-mail sdt4@cornell.edu; fax 607-255-6683.

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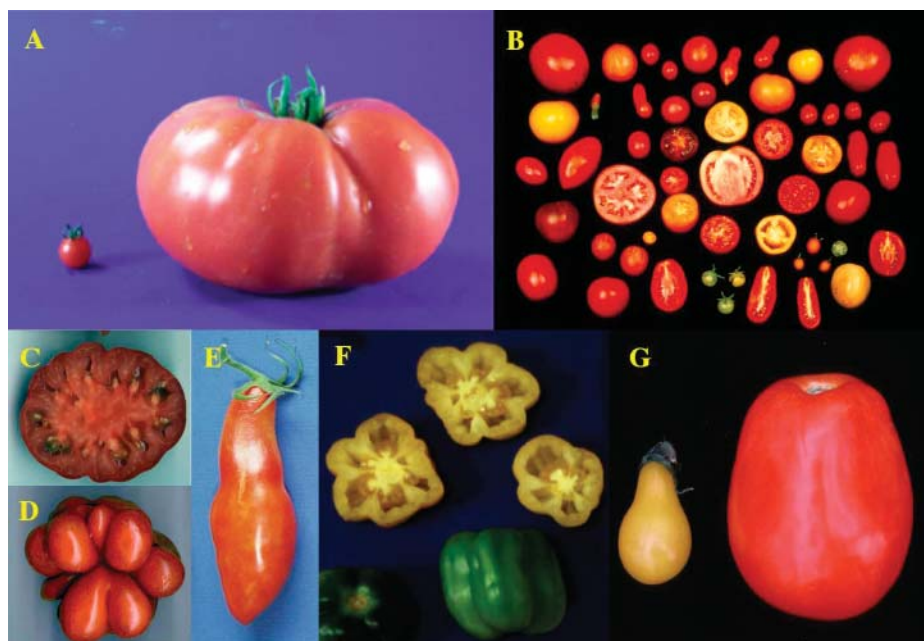


Figure 1. Collage Depicting Wide Variation in Size and Shape of Tomato Fruit.

- (A)** The large-fruited tomato cv Giant Heirloom common to modern agriculture (right), and the typical fruit of a related wild species (*L. pimpinellifolium*).
(B) The range of fruit size and shape variation in tomato.
(C) Cross-section of the fruit from a plant homozygous for a mutation at the *fasciated* locus causing multiple locules.
(D) Alternate allele of *fasciated* associated with unfused carpels.
(E) Fruit from cv Long John, which carries mutations at both the *sun* and *ovate* loci causing extremely long, narrow fruit.
(F) Bell pepper–type fruit produced by cv Yellow Stuffer.
(G) Fruit from two different cultivars homozygous for a mutation at the *ovate* locus. In the variety at left, the *ovate* mutation results in the production of fruit that are both elongated and constricted at the stem end of the fruit (hence, the pear shape). However, in the processing variety at right, the *ovate* mutation causes elongated fruit but has a much reduced effect on neck constriction. The angular or square shape of the variety at left can be attributed to the *fs8.1* locus.

higher in some varieties). The pericarp, and hence the tomato fruit, with the exception of the epidermis, is derived from the L3 layer (Szymkowiak and Sussex, 1992; Huala and Sussex, 1993). The essential descriptive aspects of tomato fruit development have been known for some time and were well summarized by Gillaspay et al. (1993). There are essentially four stages of development, which are depicted in Figure 2. These are (1) a 2- to 3-week period between floral initiation and the production of a mature flower, during which the identity, number, and shape of all floral organs are determined; (2) a period of intensive cell division that begins at anthesis and continues for ~2 weeks after fertilization; (3) a period of rapid cell expansion that begins toward the end of the cell division stage and continues until ~1 week before the onset of ripening—cells enlarge up to 20-fold during this period of development (Bergervoet et al., 1996; Cong et al., 2002) (Figure 2), which is associated with multiple rounds of endoreduplication such that cells in mature fruit are highly polysomatic, with DNA contents as high as 256C (Bergervoet et al., 1996); and (4) a ripening phase that initiates after growth has ceased and involves rapid chemical and structural changes that determine fruit aroma, color, texture, and biochemical

composition (e.g., acids and sugars) but not fruit size and shape. Because the ripening stage does not affect the overall size or shape of mature fruit, it will not be discussed further in this review. However, for a detailed discussion of this topic, the reader is referred to the article by J. Giovannoni in this issue.

SMALL AND ROUND—NATURE'S PREFERENCE?

The tomato genus, *Lycopersicon*, comprises nine species, of which only *L. esculentum* was domesticated. It is only in this cultivated species that one finds any significant phenotypic variation in fruit size and shape (Figure 1); the other eight wild species all produce fruit that are almost invariably round and small (Figure 1A). Yet, based on estimates made with DNA markers, these wild species are much more variable than their cultivated counterpart at the whole genome level. It is estimated that the genomes of tomato cultivars contain <5% of the genetic variation of their wild counterparts (Miller and Tanksley, 1990). In other words, cultivated tomatoes vary tremendously for fruit size and shape but have little genetic variation elsewhere in their genome. Conversely, wild tomatoes contain tremendous genetic

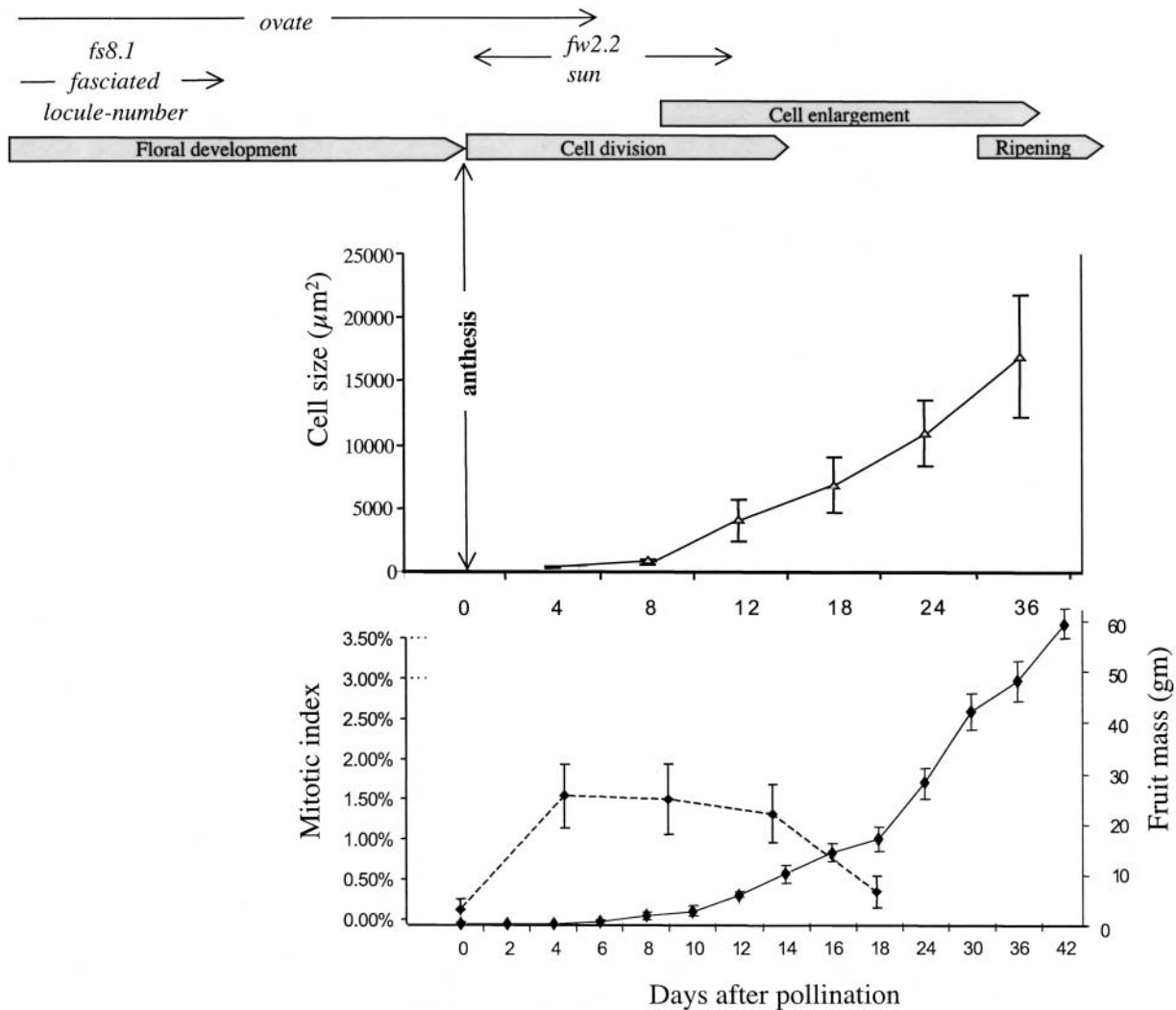


Figure 2. Stages of Tomato Fruit Development Based on Cong et al. (2002).

The developmental timing of genes known to affect tomato fruit size and shape is shown at top. Other genes are known to affect fruit size and shape, but the timing of their developmental control is currently unknown (see Table 1).

variation in their genomes but show little variation in fruit size and shape. The explanation for this seeming paradox may rest on two factors. In the wild, tomato fruit are adapted for seed dispersal. Ripening fruit, with their soft flesh and ready supply of sugars and other nutrients, attract small rodents and birds. In this regard, small fruit may provide an advantage over large fruit for dispersal because they can be readily removed, carried, and dispersed. With regard to shape, a sphere is a very economical shape for packaging seeds, and it is unlikely that alternative shapes would have provided any benefits to dispersing animals. Also, as will be discussed below, mutations that increase size and/or change shape reduce the seed yield per gram of fruit tissue, something that would likely create a selective disadvantage.

LARGE AND VARIABLE-HUMAN PREFERENCE?

Why tomato fruit became progressively larger and more variable in shape during and after domestication is a matter of conjecture. However, the benefits to humans of increased fruit size seem apparent. Less time would be required to harvest a given mass of fruit from a large-fruited genotype than from a small-fruited genotype—a fact the reader can appreciate if given the choice of harvesting a bushel of apples versus a bushel of blueberries. It also seems logical that an increase in fruit size would contribute to an increase in total yield per plant or per unit area. However, although it is true that large-fruited modern varieties yield more than their small-fruited counterparts, to my knowledge there are

no experimental data that demonstrate a cause-and-effect relationship between large fruit and higher overall yield. In fact, an experiment in which this was tested directly, using a cloned fruit size quantitative trait locus (QTL), failed to show any relationship between fruit size and total yield (Nesbitt and Tanksley; 2001; see below).

Explaining the increase in fruit shape variation is more problematic. There may be several explanations for this phenomenon. First, selection for increased fruit size may have led to changes in fruit shape attributable to pleiotropy (e.g., a mutation that increases fruit size may inherently change fruit architecture). There is good supporting evidence for this hypothesis with regard to the mutations that have led to increased fruit size through increases in carpel/locule number (Lippman and Tanksley, 2001; see below). Similarly, mutations that affect fruit shape may have greater phenotypic effects in large-fruited versus small-fruited genetic backgrounds. Several lines of experimental evidence support this notion. For example, a highly significant correlation was found between fruit size and fruit shape, such that larger fruit displayed more extreme shapes than did their small-fruited counterparts (van der Knaap and Tanksley, 2003). Additionally, a dominant allele of the *fasciated* locus (see below) manifests a very significant change in ovary shape when present in a large-fruited genotype but not in a small-fruited genotype (L. Barrero and S.D. Tanksley, unpublished results). Also, QTL mapping studies have shown that wild tomato species occasionally contain genes/alleles that, when transferred into large-fruited genotypes, change fruit shape from round to elongated or block shapes, despite the fact that the wild species bear small, nearly perfectly round fruit (Tanksley et al., 1996; Grandillo et al., 1999). Thus, selection for larger fruit may have been causally related to increased phenotypic variation in fruit shape by (1) fruit size loci having pleiotropic effects on fruit shape and/or (2) increased fruit size enabling the phenotypic expression of “hidden” fruit shape alleles that have little or no visible effect on fruit shape in small-fruited backgrounds.

Another factor that may have contributed to changes in fruit shape are the demands of modern agriculture. For example, processing tomatoes, comprising those varieties used for juice, paste, and sauces, are field grown in large acreages and, on a per unit basis, have less value than do the fresh market tomatoes commonly sold in grocery stores. The high production demand and relatively lower value of processing tomatoes led to the development of machine-harvestable varieties starting in the 1960s. Typical round-fruited tomatoes are too soft for harvest by machines, so selection for firmer-fruited varieties resulted in a shape change from round to elongated or torpedo-shaped tomatoes (often called Roma tomatoes) (Figure 1G). Recent genetic studies have shown that this change in shape and the associated increased firmness were the result of mutations in three genes (*ovate*, *sun*, and *fs8.1*), which effect changes in fruit shape through modulations of early stages of carpel development (Table 1; see below). Finally, some of the more extreme tomato fruit shapes, such as extremely long-fruited, pear-shaped, or bell pepper-shaped tomatoes, may reflect the human inclination toward valuing novelty (Figures 1E to 1G).

Most of the Major Changes in Fruit Size and Shape Can Be Attributed to a Relatively Small Number of Loci

As mentioned above, genetic studies, some dating back to the early parts of the last century, have established that tomato fruit size and shape are largely quantitatively inherited. This complexity of inheritance initially hampered efforts to understand the processes that control fruit size and shape at the molecular level. The advent of marker-assisted QTL mapping/cloning techniques has now made this a tractable problem. During the past 15 years, a number of QTL studies have been conducted involving crosses between wild tomatoes and cultivated tomatoes of various sizes and shapes (Grandillo et al., 1999). The conclusion of this research is that ~30 QTLs account for most of the variation in both tomato fruit size and shape. However, these loci are not equal in the magnitude of their effects. Based on all of the genetic/mapping studies conducted to date, it is estimated that <10 loci, mapping to 7 of the 12 chromosomes, account for the majority of the changes in size and shape associated with tomato domestication/agriculture (Grandillo et al., 1999) (Table 1). Understanding the function of these genes and the manner in which mutations in these genes perturb developmental processes is key to our understanding of fruit development and evolution. Thus, for the remainder of this article, I concentrate on summarizing what is known about the developmental and, in some cases, cellular/molecular processes that are controlled by these major tomato fruit size and shape loci.

Key Loci Controlling Fruit Size

Mutation(s) in six loci seem to have been essential in transforming the small, inconspicuous berries of wild tomatoes to the extremely large fruit now associated with modern cultivars (Figure 1, Table 1). A summary of our knowledge of these loci follows.

fw1.1, *fw2.2*, *fw3.1*, and *fw4.1*

These four loci were first identified as QTLs in crosses between small-fruited wild tomatoes and their large-fruited cultivated counterparts (Grandillo et al., 1999). They share several features in common. First, they have been confirmed by multiple mapping experiments and/or studies of nearly isogenic lines. Second, natural allelic variation at these loci has a major impact on fruit size and can change the final fruit mass by as much as 30%. Third, the effects of these loci appear to be largely confined to fruit mass and not fruit shape, with the possible exception of *fw3.1* (van der Knaap et al., 2002; van der Knaap and Tanksley, 2003). To date, *fw2.2* is the only one of these loci that has been cloned and studied at the molecular level. The knowledge gleaned from molecular studies of *fw2.2* is summarized below.

It is believed that a mutation(s) in the *fw2.2* locus on chromosome 2 was one of the first steps on the road to domestication. Natural genetic variation at this locus alone can change the size of fruit by up to 30% (Frary et al., 2000). All wild tomatoes tested to date have “small-fruit” alleles at this locus, whereas all cultivated tomatoes tested are fixed for “large-fruit” alleles. Developmental studies with nearly isogenic lines have

Table 1. List of Key Loci Controlling Size and Shape Variation in Tomato

Locus/QTL	Marker (Chromosome) ^a	Cellular/Molecular Function	Protein Type	Cause of Allelic Variation	Homologous Gene with a Similar Effect in Other Fruit-Bearing Species	Alias(es)
<i>fw1.1</i>	TG58 (1)	Unknown	Unknown	Unknown		
<i>fw2.2</i>	<i>fw2.2</i> ^b	Negative regulator of cell division, possibly involved in cell-to-cell communication	Novel, membrane-spanning protein	Quantitative and temporal changes in gene expression	Orthologous gene maps to the most significant fruit weight QTL in eggplant and may correspond to a major fruit weight QTL in pepper	
<i>fw3.1</i>	SSR111 (3)	Unknown	Unknown	Unknown	Colocalizes with a major fruit weight QTL in pepper	
<i>fw4.1</i>	SSR450 (4)	Unknown	Unknown	Unknown		
<i>locule-number</i>	cLEC7H4 (2)	Unknown	Unknown	Unknown		
<i>fasciated</i>	TG105A (11)	Unknown	Unknown	Unknown		<i>fw11.3, fs11.a</i>
<i>ovate</i>	<i>ovate</i> ^b	Possibly involved in determining the polarity of cell division in early carpel development	Putative regulatory protein localized in the nucleus	Premature stop codon in an exon associated with a mutant phenotype	Orthologous gene maps to a fruit shape QTL in eggplant	
<i>sun</i>	CT52 (7)	Unknown	Unknown	Unknown		<i>fs7.a</i>
<i>fs8.1</i>	SSR244 (8)	Unknown	Unknown	Unknown	Colocalizes with a major fruit shape QTL in pepper	

Phenotypic Effect	Gene Action (Large Fruit or Nonround Fruit Allele)	Pleiotropic Effects	Frequency/Distribution in Germplasm	Developmental Stage/Process Affected	Key Reference
Fruit mass	Additive	None known	Most cultivated tomatoes carry a large-fruit allele at this locus	Unknown	Grandillo et al., 1999; S.D. Tanksley, unpublished results
Fruit mass	Additive	Increased sepal length	Most cultivated tomatoes carry a large-fruit allele at this locus	Cell division in carpels from anthesis through ~2 weeks after anthesis	Frery et al., 2000; Ben Chaim et al., 2001; Cong et al., 2002; B. Cong, unpublished results; Doganlar et al., 2002
Fruit mass	Additive	None known	Many wild species carry a small fruit allele	Unknown	Grandillo et al., 1999; Ben Chaim et al., 2001
Fruit mass	Additive	None known	Many wild species and some cherry tomatoes carry a small-fruit allele	Unknown	Grandillo et al., 1999; Doganlar et al., 2000; S.D. Tanksley, unpublished results
Increase in the number of carpels/locules, resulting in larger and more oblate-shaped fruit	Additive	Concomitant changes in the number of floral organs (e.g., sepals, petals, stamens)	Largely restricted to multilocular, large, fresh market tomato varieties	Early carpel development, at least 2 weeks before anthesis	Lippman and Tanksley, 2001
Increase in the number of carpels/locules, resulting in larger and more oblate-shaped fruit	Largely recessive	Concomitant changes in the number of floral organs (e.g., sepals, petals, stamens)	Largely restricted to multilocular, large, fresh market tomato varieties	Early carpel development, at least 2 weeks before anthesis	Lippman and Tanksley, 2001
Asymmetric growth in the longitudinal axis of the carpel, resulting in pear- or torpedo-shaped fruit	Recessive/partially recessive	None known	Largely restricted to a subset of pear-shaped or elongated (Roma) tomato varieties	Early carpel development, at least 2 weeks before anthesis	Doganlar et al., 2002; Liu et al., 2002
Asymmetric growth in the longitudinal axis of fruit soon after anthesis	Additive	None known	Found in some tomato cultivars with elongated or very elongated fruit	Affects growth phase immediately after anthesis	van der Knaap and Tanksley, 2001
Lobe-shaped locules resulting in blocky fruit shape	Recessive/partially recessive	None known	Largely modern cultivars with blocky fruit shape, especially modern processing tomato cultivars	Early carpel development, at least 2 weeks before anthesis	Ku et al., 2000; Ben Chaim et al., 2001; van der Knaap et al., 2003

^a Position of the locus on the genetic map based on the most closely linked marker (for map, see <http://www.sgn.cornell.edu/>).

^b The corresponding gene has been cloned and localized directly on the genetic map.

shown that the changes in fruit size associated with *fw2.2* are most obvious during the latter stages of fruit development (Nesbitt and Tanksley, 2001; Cong et al., 2002; Liu and Tanksley, 2003). However, histological studies of the same isogenic lines trace the causes of fruit size changes to earlier stages of fruit development. Large-fruit alleles of *fw2.2* are associated with a higher mitotic index (especially in cortical tissue) during the cell

division stage just after anthesis (Cong et al., 2002) (Figure 2). Cloning of *fw2.2* has shown that this locus codes for a negative repressor of cell division, with activity confined largely to the cell division phase of fruit development (Frery et al., 2000; Cong et al., 2002). *FW2.2* is homologous with other plant proteins, but none of them has a known biological function. *FW2.2* has two predicted transmembrane-spanning domains, a finding consistent

with cell bombardment studies with GREEN FLUORESCENT PROTEIN-FW2.2 fusion constructs, which show the protein to be localized to the cell membrane (B. Cong and S.D. Tanksley, unpublished results). The pathway in which FW2.2 functions in controlling cell division is currently unknown, but the working hypothesis is that it is involved in the cell-to-cell communication/coordination of cell division.

An interesting feature of *fw2.2* is that the mutations associated with changes from small to large fruit are in the promoter, rather than in the coding portion, of the gene. These changes in the promoter are associated with lower total transcript levels during the cell division phase of fruit development as well as a shift in the timing of expression (Cong et al., 2002). Changes in gene regulation, rather than protein function, have long been hypothesized as a major mode of evolutionary change, especially concerning morphological differentiation. In this regard, *fw2.2* is one of a growing number of examples in which natural variation associated with morphological changes can be traced to regulatory mutations (Wang et al., 1999; Cong et al., 2002). It also is worth noting that, although *fw2.2* has a major impact on the outcome of fruit development, the gene itself is expressed at very low levels and for only a short time (Cong et al., 2002). In fact, despite the availability of a tomato EST database containing >150,000 ESTs, many derived from cDNA libraries of developing fruit (<http://www.sgn.cornell.edu/>), no EST exists in the database for *fw2.2* and no cDNA clone has been identified for this gene (S.D. Tanksley, unpublished results).

Finally, with regard to other aspects of development, the mutations in *fw2.2* that cause changes in fruit size appear to affect only fruit size. To date, no significant changes in plant architecture, fruit shape, or seed size/number or yield are associated with natural allelic variation at *fw2.2* (Nesbitt and Tanksley, 2001; Liu and Tanksley, 2003).

fasciated* and *locule-number

Genes such as *fw2.2* affect fruit mass and do not exert major effects on the overall structural organization or shape of fruit. However, final fruit mass can be affected by changes in the shape and architecture of the ovaries within the flower. Wild tomato species, and many cultivated varieties, produce flowers with a gynoeceum containing two to four carpels. After fertilization, each carpel develops into a locule in the fruit. However, some varieties produce fruit with more locules, often resulting in larger, wider fruit (Lippman and Tanksley, 2001; van der Knaap and Tanksley, 2003) (Figure 1C).

Two loci, *fasciated* (chromosome 11) and *locule-number* (chromosome 2), have been identified as causing changes in fruit size via changes in the number of carpels in the flower (Table 1, Figure 1C). All of the very large-fruited, multilocular fresh market tomatoes tested to date carry mutations in one or both of these genes (Lippman and Tanksley, 2001; L. Barrero and S.D. Tanksley, unpublished results). The effect of naturally occurring mutations at the *fasciated* locus are more radical than those at the *locule-number* locus. Plants carrying mutant alleles at *fasciated* can produce >15 locules. Moreover, one mutant allele of *fasciated* is associated not only with more carpels but also with unfused carpels (Figure 1D). Plants with the *fasciated* mutation

also show concomitant increases in the number of other floral organs, including sepals, petals, and stamens. Developmental studies using isogenic lines have shown that phenotypic differences between wild-type and *fasciated* stocks appear very early in floral development (L. Barrero and S.D. Tanksley, unpublished results).

Most multilocular, fresh market tomato varieties carry the *fasciated* mutation. However, the production of the extremely large fruit (>500 g) characteristic of some varieties apparently requires the presence of not only the *fasciated* mutation but also the *locule-number* mutation. When present together, these mutations interact epistatically to produce fruit with an exceptionally high locule number (and hence greater size) (Lippman and Tanksley, 2001; L. Barrero and S.D. Tanksley, unpublished results). *fasciated* and *locule-number* differ in one significant respect. Genetic studies have shown that the increase in carpel number associated with mutations at *fasciated* behaves in a recessive manner, suggesting that such mutations render a loss-of-function mutant. However, *locule-number* alleles normally behave in an additive manner, raising the possibility that the mutation(s) causing modulations in *locule-number* are regulatory rather than structural in nature. The nature of the allelic variations and developmental pathways in which *fasciated* and *locule-number* genes act cannot be understood fully until both of these key loci are isolated.

Key Loci Controlling Fruit Shape

As mentioned above, there is not a complete separation between the loci controlling fruit size and those controlling fruit shape. The organ-determining genes *fasciated* and *locule-number* affect both the final size and shape of the fruit. However, the other major fruit size loci, *fw1.1*, *fw2.2*, *fw3.1*, and *fw4.1*, exert their effects largely on fruit growth, resulting in changes in size with little if any changes in shape. Similarly, there are three major loci that modulate fruit shape yet have a minimal effect on fruit size. These loci are *ovate* (chromosome 2), *sun* (chromosome 8), and *fs8.1* (chromosome 8) (Table 1). A brief summary of our current knowledge of each of these loci follows.

ovate

In the early 1900s, breeders reported the segregation of a locus conditioning pear-shaped tomato fruit characterized by a constriction at the stem end of the fruit (Hendrick and Booth, 1907; Price and Drinkard, 1908). During this same period, others reported a similar locus causing oblate- to oval-shaped fruit. It was shown subsequently that these genes were likely allelic, and the corresponding locus was referred to as *ovate* (Lindstrom, 1928, 1929). Nearly 75 years lapsed before further research was conducted on the *ovate* locus, when Ku et al. (1999) constructed nearly isogenic lines for *ovate* and showed that the single locus could account for both the pear shape and the elongated fruit shape. The *ovate* gene was cloned recently and shown to correspond to a new class of nucleus-localized, putative regulatory proteins (Liu et al., 2002). The gene is expressed from the early stages of floral development (<10 days after pollination) through the first 2 weeks after anthesis (Liu et al., 2002) (Figure 2).

The mutation associated with a change from round to elongated or pear-shaped fruit creates a premature stop codon in the second exon. This putative loss-of-function mutation is consistent with the recessive behavior of the elongated or pear-shaped allele in genetic studies (Liu et al., 2002).

The origin of the spontaneous mutation in *ovate* is unknown. However, a survey of diverse germplasm has shown but a single mutant allele of *ovate*, and its distribution is restricted to the subset of germplasm with elongated or pear-shaped fruit (J. Liu and S.D. Tanksley, unpublished results). A curious feature of *ovate* is that the mutation is not associated with a single phenotype. In some genetic backgrounds, the mutation leads to elongated fruit with highly constricted neck growth reminiscent of pears (Figure 1G). However, in other backgrounds, little or no neck constriction is observed and the degree of fruit elongation is not so pronounced, suggesting that the *ovate* locus interacts with other, as yet unknown loci in the genome (Figure 1G).

sun

During our genetic studies of the *ovate* locus, we observed some processing tomato varieties with elongated fruit shape that did not carry the *ovate* mutation. QTL mapping with one of these stocks revealed the presence of a second major fruit shape locus on chromosome 7; this locus was named *sun* after the variety Sun 1642, in which the locus was first identified (van der Knaap and Tanksley, 2001). Although allelic variation at both *ovate* and *sun* can cause elongated fruit shape, the two loci differ in some important genetic, morphological, and developmental respects. First, *sun* causes uniform elongation in both longitudinal directions, such that the fruit maintain a bilateral symmetry, whereas *ovate* usually results in asymmetric elongation, such that the stem end of the fruit is more exaggerated than the blossom end. Also, neck constriction or pear shape has never been associated with this locus (van der Knaap et al., 2002). Second, developmental studies have shown that the changes in fruit shape associated with *sun* occur after pollination, during the cell division stage of fruit development (van der Knaap and Tanksley, 2001). *ovate*, in contrast, acts much earlier during floral development. Finally, alleles of *sun* interact in an additive manner, raising the possibility that mutations that lead to elongated fruit are regulatory rather than structural in nature (van der Knaap and Tanksley, 2001). The *sun* locus has been localized to a 0.2-centimorgan interval comprising 66 kb, and it seems likely that the molecular nature of this gene will be revealed soon (E. van der Knaap, unpublished results).

fs8.1

The popular notion of the “square tomato” has been around for several decades and is not based entirely on folklore. In the early 1960s, efforts began in earnest to develop machine harvesting for tomatoes, with particular emphasis on processing tomatoes, which are grown over large acreages. One of the practical hurdles was that tomato fruit were too soft to harvest with any of the harvesting machines being devised by agricultural engineers. If a machine could not be designed that would not damage the tomato fruit, then tomato fruit needed to be designed that could

withstand machine harvest. That was exactly what happened, and the results eventually led to today’s machine-harvestable “square” tomato (Figure 1G). These early processing tomatoes had two noticeable changes in fruit shape. First, they were no longer round but rather slightly elongated. Second, the individual carpels took on a more bulbous, extended morphology, giving the fruit the appearance of being block- or square-shaped (Figure 1G). It was presumably this latter change that improved the endurance of the fruit during mechanical harvesting.

Several QTL mapping studies have shown that a single major locus, *fs8.1*, is responsible for both the blocky and slightly elongated appearance of processing tomatoes (Grandillo et al., 1996). Developmental studies with nearly isogenic lines have shown that the change in fruit shape attributed to *fs8.1* begins very early in floral/carpel development (Ku et al., 2000), a characteristic shared with *ovate*, *fasciated*, and *locule number*. By anthesis, the carpels have already assumed the blocky, elongated shape that will characterize to the mature fruit. Thus, it is predicted that *fs8.1* functions mostly or entirely during early floral development and has little if any activity after anthesis (Ku et al., 2000) (Figure 2).

The Combined Effects of the Major Fruit Size and Shape Loci Account for Most of the Extreme Variations in Fruit Size and Shape in Modern Tomatoes

Tomatoes come in a bewildering variety of sizes and shapes (Figure 1B). The size and shape loci described above can individually modulate the size and/or shape of tomato fruit. However, it has been unclear whether these loci can account for the more extreme fruit sizes and shapes. For example, some heirloom varieties produce fruit of up to 1000 g (Figure 1A). Likewise, varieties exist that produce fruit that look nearly indistinguishable from bell pepper fruit (Figure 1F) or that are extremely long and narrow (Figure 1E). It is interesting that several genetic studies involving extreme fruit size or shape varieties have suggested the combined action of two or more of the nine loci described in this review. For example, the heirloom variety Giant Heirloom owes its immense fruit size to the combined action of *fw1.1*, *fw2.2*, *fw3.1*, *locule-number*, and *fasciated* (Figure 1A) (Lippman and Tanksley, 2001). The most significant loci contributing to the highly elongated fruit of cv Long John are *ovate* and *sun* (Figure 1E) (van der Knapp et al., 2002). Likewise, the bell pepper-shaped fruit produced by the variety Yellow Stuffer can be traced largely to the effects of *fw2.2*, *fs8.1*, and *locule-number* (Figure 1F) (van der Knapp and Tanksley, 2003). These combined results suggest that the evolution of tomato size and shape from the small, round fruit of wild tomatoes was attributable to mutations at only a few loci and that the nine loci described here were among the most significant contributors.

Did Other Fruit-Bearing Crops Evolve Domesticated Fruit Types through Mutations in Orthologs of the Same Genes as Tomato?

Much of this review has been devoted to addressing the question of which key loci triggered major changes in tomato fruit

morphology during domestication. However, a parallel and equally interesting question is whether similar morphological changes that occurred during the domestication of other fruit crops were attributable to mutations in orthologous genes or common developmental pathways. As described above, a number of phylogenetically diverse plant taxa have been domesticated for the production of edible fruit. Moreover, in most instances, domestication resulted in both a dramatic increase in fruit size and enhanced variation in fruit shape. If we can elucidate the genes and developmental pathways that were perturbed to create large edible fruit in these different plant taxa, we may begin to answer this second question. In this regard, there have been parallel QTL mapping studies for fruit size and shape in multiple fruit-bearing solanaceous crops, such as tomato, eggplant, and pepper (Ben Chaim et al., 2001; Doganlar et al., 2002). Because these studies used common, orthologous genetic markers, it has been possible to identify fruit size/shape QTLs that are likely attributable to mutations in orthologous genes. Other potential commonalities between tomato and pepper fruit size and shape are summarized in Table 1. Perhaps the most striking example of common genes/pathways is observed in comparisons of tomato and eggplant, both of which evolved from small-fruited wild ancestors. A recent QTL mapping study involving a large-fruited commercial eggplant variety and a small-fruited wild ancestor has identified the ortholog of *fw2.2* as being the most significant locus accounting for increases in fruit size in the creation of domesticated eggplant (Doganlar et al., 2002).

Nevertheless, it is difficult to draw any final conclusions about the degree to which homologous genes and parallel pathways were involved in the control of fruit size and shape in different fruit-bearing plants. However, the facts that fewer than 10 major loci account for most of the variation in fruit size and shape in tomato and that at least some of these genes appear to underlie similar variations in other solanaceous plants suggest an underlying commonality in this aspect of plant development and evolution.

WHAT'S NEXT?

Although significant progress has been made in identifying the loci involved in creating the large and variably shaped fruit that are characteristic of modern tomatoes, we are just beginning to understand how the proteins encoded by these genes actually modulate fruit size and shape. The first two shape and size QTLs cloned (*fw2.2* and *ovate*) code for novel, plant-specific proteins. Although similar proteins can be found in other plant species (including those that do not produce edible fruit, such as *Arabidopsis* and rice), none of these homologs has a known function. The challenge for at least the next decade will be not only to clone the remaining genes that underlie size and shape variation but, even more importantly, to elucidate the molecular and cellular pathways in which these proteins function. An important aspect of this quest for function will be the need to understand the degree to which these pathways are shared among both fruit-bearing and non-fruit-bearing crop species. Fortunately, most of the tools to address these questions in tomato and other model plant species are now in place.

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REFERENCES

- Balbi, V., and Lomax, T.L. (2003). Regulation of early tomato fruit development by the *Diageotropica* gene. *Plant Physiol.* **131**, 725–735.
- Banerjee, M.K., and Kalloo, G. (1989). The inheritance of earliness and fruit weight in crosses between cultivated tomatoes and two species of *Lycopersicon*. *Plant Breed.* **102**, 148–152.
- Ben Chaim, A., Paran, I., Grube, R.C., Jahn, M., van Wijk, R., and Peleman, J. (2001). QTL mapping of fruit-related traits in pepper (*Capsicum annuum*). *Theor. Appl. Genet.* **102**, 1016–1028.
- Bergervoet, J.H.W., Verhoeven, H.A., Luun, J.W., Gilissen, J.W., and Bino, R.J. (1996). High amounts of nuclear DNA in tomato (*Lycopersicon esculentum* Mill.) pericarp. *Plant Sci.* **116**, 141–145.
- Cong, B., Liu, J., and Tanksley, S.D. (2002). Natural alleles at a tomato fruit size quantitative trait locus differ by heterochronic regulatory mutations. *Proc. Natl. Acad. Sci. USA* **99**, 13606–13611.
- Doganlar, S., Frary, A., Daunay, M.-C., Lester, R.N., and Tanksley, S.D. (2002). Conservation of gene function in the Solanaceae as revealed by comparative mapping of domestication traits in eggplant. *Genetics* **161**, 1713–1726.
- Doganlar, S., Tanksley, S., and Mutschler, M. (2000). Identification and molecular mapping of loci controlling fruit ripening time in tomato. *Theor. Appl. Genet.* **100**, 249–255.
- Frary, A., Nesbitt, T.C., Frary, A., Grandillo, S., van der Knaap, E., Cong, B., Liu, J., Meller, J., Elber, R., Alpert, K., and Tanksley, S. (2000). Cloning and transgenic expression of *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit. *Science* **289**, 85–87.
- Gillaspy, G., Ben-David, H., and Gruissem, W. (1993). Fruits: A developmental perspective. *Plant Cell* **5**, 1439–1451.
- Grandillo, S., Ku, H.-M., and Tanksley, S.D. (1996). Characterization of *fs8.1*, a major QTL influencing fruit shape in tomato. *Mol. Breed.* **2**, 251–260.
- Grandillo, S., Ku, H.-M., Tanksley, S.D. (1999). Identifying loci responsible for natural variation in fruit size and shape in tomato. *Theor. Appl. Genet.* **99**, 978–987.
- Hendrick, U.P., and Booth, N.O. (1907). Mendelian characters in tomatoes. *Proc. Am. Soc. Hortic. Sci.* **5**, 19–24.
- Huala, E., and Sussex, I.M. (1993). Determination and cell interactions in reproductive meristems. *Plant Cell* **5**, 1157–1165.
- Jenkins, J.A. (1948). The origin of the cultivated tomato. *Econ. Bot.* **2**, 379–392.
- Ku, H.-M., Doganlar, S., Chen, K.-Y., and Tanksley, S. (1999). The genetic basis of pear-shaped tomato fruit. *Theor. Appl. Genet.* **9**, 844–850.
- Ku, H.-M., Grandillo, G., and Tanksley, S.D. (2000). *fs8.1*, a major QTL, sets the pattern of tomato carpel shape well before anthesis. *Theor. Appl. Genet.* **101**, 873–878.
- Lindstrom, E.W. (1928). Linkage of size, shape and color genes in *Lycopersicum*. *Verh. 5th Int. Kong. Vererb. Ber.* **2**, 1031–1057.
- Lindstrom, E.W. (1929). Fruit-size and shape genes on the first chromosome of the tomato. *Iowa Acad. Sci.* **36**, 189–190.

- Lippman, Z., and Tanksley, S.D.** (2001). Dissecting the genetic pathway to extreme fruit size in tomato using a cross between the small-fruited wild species *L. pimpinellifolium* and *L. esculentum*, var. Giant Heirloom. *Genetics* **158**, 413–422.
- Liu, J., and Tanksley, S.D.** (2003). Generation and analysis of an artificial gene dosage series in tomato to study the mechanisms by which the cloned QTL *fw2.2* controls fruit size. *Plant Physiol.* **132**, 292–299.
- Liu, J., Van Eck, J., Cong, B., and Tanksley, S.D.** (2002). A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. *Proc. Natl. Acad. Sci. USA* **99**, 13302–13306.
- MacArthur, J.W., and Butler, L.** (1938). Size inheritance and geometric growth processes in the tomato fruit. *Genetics* **23**, 253–268.
- Miller, J.C., and Tanksley, S.D.** (1990). RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theor. Appl. Genet.* **80**, 437–448.
- Nesbitt, T.C., and Tanksley, S.D.** (2001). *fw2.2* directly affects the size of developing tomato fruit, with secondary effects on fruit number and photosynthate distribution. *Plant Physiol.* **127**, 575–583.
- Price, H.L., and Drinkard, A.W.** (1908). Inheritance in tomato hybrids. *Va. Agric. Exp. Stn. Bull.* **177**, 15–53.
- Rick, C.M.** (1995). Tomato – *Lycopersicon esculentum* (Solanaceae). In *Evolution of Crop Plants*, J. Smartt and N.W. Simmonds, eds (London: Longman Scientific & Technical), pp. 452–457.
- Smartt, J., and Simmonds, N.W.**, eds (1995). *Evolution of Crop Plants*. (London: Longman Scientific & Technical).
- Szymkowiak, E.J., and Sussex, I.M.** (1992). The internal meristem layer (L3) determines floral meristem size and carpel number in tomato periclinal chimeras. *Plant Cell* **4**, 1089–1100.
- Tanksley, S.D., Grandillo, S., Fulton, T.M., Zamir, D., Eshed, Y., Petiard, V., Lopez, J., and Beck-Bunn, T.** (1996). Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. *Theor. Appl. Genet.* **92**, 213–224.
- van der Knapp, E., Lippman, Z.B., and Tanksley, S.D.** (2002). Extremely elongated tomato fruit controlled by four quantitative trait loci with epistatic interactions. *Theor. Appl. Genet.* **104**, 241–247.
- van der Knaap, E., and Tanksley, S.D.** (2001). Identification and characterization of a novel locus controlling early fruit development in tomato. *Theor. Appl. Genet.* **103**, 353–358.
- van der Knaap, E., and Tanksley, S.D.** (2003). The making of a bell pepper-shaped tomato fruit: Identification of loci controlling fruit morphology in Yellow Stuffer tomato. *Theor. Appl. Genet.* **107**, 139–147.
- Wang, R.-L., Stec, A., Hey, J., Lukens, L., and Doebley, J.** (1999). The limits of selection during maize domestication. *Nature* **398**, 236–239.