The Making of a Compound Inflorescence in Tomato and Related Nightshades

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Introduction

A striking manifestation of plant evolution is observed in the diverse branching and patterning of inflorescences, which are the shoots that bear flowers [1,2]. Inflorescences are derived from the growth of dome-shaped groups of pluripotent cells called apical meristems. Apical meristems first produce leaves, and upon flowering induction, they produce inflorescence meristems that transition to floral meristems, which produce flowers. Extensive variation in inflorescence complexity is found in the nightshade (Solanaceae) family, where flowering marks the end of main shoot growth, and vegetative aerial growth is renewed from axillary meristems in a perennial growth system known as “sympodial” [3–5]. The simplest Solanaceae inflorescence is a solitary flower, represented by pepper (Capsicum annum) in Figure 1A. Tomato (Solanum lycopersicum), on the other hand, generates a few-flowered inflorescence organized in a zigzag branch (Figure 1B), but there are three classical mutants called compound inflorescence (s) and anantha (an) (Figure 1F and 1G), and falsiflora (fa) (Figure 1H) that bear highly branched inflorescences resembling wild Solanacea species like S. crispum (Figure 1C) [6–8]. These similarities suggest that branching complexity may arise from tuning a common underlying developmental program. We set out to begin to unravel the basis of Solanaceae inflorescence diversity using these mutants whose variation ranges from branched inflorescences that produce hundreds of fertile flowers as seen in s [6], to the branching shoots of an that terminate in cauliflower-like tissue [7], to the leafy inflorescences of fa, which is defective in the tomato ortholog of LEAFY (LFY) [9].

Results

Development of Normal and Ramified Inflorescence Types

The tomato plant is a compound shoot formed from reiterated sympodial shoot units (SYM) that arise from vegetative meristems that produce three leaves before terminating with an inflorescence [10]. The tomato inflorescence is also a compound shoot, which is condensed, consisting of sequential one-nodal inflorescence sympodial units (ISUs) each terminated by a single flower [11]. During early inflorescence development, individual ISUs developed in a progression of two phases. In the first phase, a sympodial inflorescence meristem (SIM), which was distinct from a SYM because it formed within the inflorescence itself, arose and produced a new SIM on its side before differentiating into a floral meristem (FM) in a second phase. These events created the first ISU and the SIM of the second ISU (Figure 2 and Figure S1). This pattern reiterated as subsequent SIMs developed perpendicularly to one another, producing a zigzag pattern of flower initiation (Figure 2A). In an and fa mutants, the primary meristems failed to become flowers, remained indeterminate, and repeatedly initiated secondary SIMs that, themselves, repeatedly produced SIMs (Figure 2 and Figure S1). s was more asynchronous, as SIMs eventually transitioned to flowers after producing 2–4 axillary SIMs in a variable, environment-dependent manner (Figure 2 and Figure S1).

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Variation in the branching of plant inflorescences determines flower number and, consequently, reproductive success and crop yield. Nightshade (Solanaceae) species are models for a widespread, yet poorly understood, program of eudicot growth, where short side branches are initiated upon floral termination. This “sympodial” program produces the few-flowered tomato inflorescence, but the classical mutants compound inflorescence (s) and anantha (an) are highly branched, and s bears hundreds of flowers. Here we show that S and AN, which encode a homeobox transcription factor and an F-box protein, respectively, control inflorescence architecture by promoting successive stages in the progression of an inflorescence meristem to floral specification. S and AN are sequentially expressed during this gradual phase transition, and the loss of either gene delays flower formation, resulting in additional branching. Independently arisen alleles of s account for inflorescence variation among domesticated tomatoes, and an stimulates branching in pepper plants that normally have solitary flowers. Our results suggest that variation of Solanacea inflorescences is modulated through temporal changes in the acquisition of floral fate, providing a flexible evolutionary mechanism to elaborate sympodial inflorescence shoots.
Author Summary

Among the most distinguishing features of plants are the flower-bearing shoots, called inflorescences. Despite a solid understanding of flower development, the molecular mechanisms that control inflorescence architecture remain obscure. We have explored this question in tomato, where mutations in two genes, ANANATHA (AN) and COMPOUND INFLORESCENCE (S), transform the well-known tomato "vine" into a highly branched structure with hundreds of flowers. We find that AN encodes an F-box protein ortholog of a gene called UNUSUAL FLORAL ORGANS that controls the identity of floral organs (petals, sepals, and so on), whereas S encodes a transcription factor related to a gene called WUSCHEL HOMEBOX 9 that is involved in patterning the embryo within the plant seed. (F-box proteins are known for marking other proteins for degradation, but they can also function in hormone regulation and transcriptional activation) Interestingly, these genes have little or no effect on branching in inflorescences that grow continuously (so-called "indeterminate" shoots), as in Arabidopsis. However, we find that transient sequential expression of S followed by AN promotes branch termination and flower formation in plants where meristem growth ends with inflorescence and flower production ("determinate" shoots). We show that mutant alleles of s dramatically increase branch and flower number and have probably been selected for by breeders during modern cultivation. Moreover, the single-flower inflorescence of pepper (a species related to tomato, within the Solanaceae) is based on an s-classic allele, demonstrating that s is mutated in this gene (Figure 3) [6]. However, it was also possible that one or more of these lines arose independently, generating a mutation in the same nucleotide as s-classic. To address this question, we sequenced the coding region of all 22 lines and un-branched controls and found that all were identical except CC5721. Interestingly, this line carried four single-nucleotide polymorphisms (SNPs) that were shared with at least one un-branched variety, indicating that the s mutation in CC5721 may have arisen independently from a genetically distinct progenitor line (GenBank accessions FJ190665, FJ190666, and FJ190667). Two pieces of evidence lend support to this claim. Firstly, the four SNPs were distributed close (all within 1,000 bp) to the s lesion. Secondly, we sequenced a short segment of DNA from the tightly linked bacterial artificial chromosome (BAC) 298N3 and found that CC5721 had six SNPs and a 9-bp insertion-deletion (indel) that distinguished it from the other 21 domesticated types carrying s-classic (Figure 3b) (GenBank accessions FJ215691 and FJ215692). Still, in the absence of a geographic distribution of haplotypes, we cannot exclude a remote possibility that CC5721 arose as a result of an intragenic recombination between s-classic and an unbranched variety. Regardless, at least three independently arisen alleles of s (s-classic, s-multiflora, and Rose Quartz Multiflora) are responsible for a major portion of the diversity in tomato inflorescence architecture.

Although the branching effects were similar between the three mutants, floral phenotypes were not. Mutants of fa were primarily vegetative, producing numerous leaves that developed early as primordia coming off the flanks of meristems (Figure 1H and Figure S1K). Mutants of an, on the other hand, produced leaf primordia mixed with other tissue that at maturity resembled modified sepals or bracts (Figure 1F and 1G). It is interesting that s mutants maintained the capacity to produce normal flowers, indicating a reduced role in the flower relative to the SIM, although occasionally we observed some leaf-like primordia (Figure S1F). Thus, beyond distinctions in controlling floral organ identity (Figure 1), s, an, and fa mutants exhibit delayed ISU maturation, resulting in additional branches through the ongoing initiation of lateral SIMs. Notably, SIM branching in diverse Solanaceae is based on an s-like program, as seen in early inflorescence development of S. crispum (Figure 2D and Figure S2). This suggests that delays in floral termination (perhaps mediated by S, or the genetic pathway that S defines) provide a developmental framework for the modulation of sympodial branching in the Solanaceae.

**compound inflorescence** Encodes a Wuschel-Homebox Transcription Factor and Is Responsible for a Major Portion of Inflorescence Variation in Domesticated Tomatoes

To identify the genes responsible for these phenotypes, s and an were localized to linked regions of chromosome 2, and s was positionally cloned using a remarkable level of multi-genome synteny between the eudicot species poplar (Populus trichocarpa), Barrel Medic (Medicago truncatula), and grape (Vitis vinifera) (Figure 3). Several genes were shared in a short chromosomal segment ranging from 105–140 kb, and aligning these regions revealed three transcription factors: two AP2-like genes and a WUSCHEL-homeobox (WOX) that each co-segregated with s. Sequencing of all three genes revealed independent point mutations in the WOX gene from two alleles of s (s-classic and s-multiflora), and Southern blot analysis showed chromosomal changes in an additional allele (s-n5568), demonstrating that s is mutated in this gene (Figure 3 and Figure S3B and S4) (GenBank (http://www.ncbi.nlm.nih.gov/Genbank) accessions FJ190663 and FJ190664). To our knowledge, this is the first example of gene identification using multi-genome synteny among four eudicot species. Our data suggest that an even greater level of synteny remains to be discovered, and that non-model species will realize similar benefits as more genomes are sequenced.

WOX proteins share homology with the meristem maintenance gene WUSCHEL and are plant-specific transcription factors [12]. Among 14 WOX genes in Arabidopsis, S is most similar to WOX9/STIMPY (STIP) that functions with WOX8/STIPL to regulate embryonic patterning [13]. In contrast, we have found that S is a major determinant of inflorescence architecture in tomato. In the context of a European Solanaceae project (Eu-Sol), we established and phenotyped a collection of more than 6000 domesticated tomato varieties for various traits (Materials and Methods; https://www.eu-sol.wur.nl). The power of such a large germplasm resource resides in the fact that extensive natural allelic variation with both qualitative and quantitative effects has been selected and maintained since tomato was first domesticated [14]. Thus, these varieties provide a complement to the stronger, mostly deleterious effects, of alleles derived from artificial mutagenesis [8]. Among the 6,000 tomato lines, we identified 23 accessions with highly branched inflorescences and all were allelic to s. Surprisingly, 22 of these lines carried the s-classic allele of the original mutant described 100 years ago, indicating that early breeders were positively selecting this mutant, probably for aesthetic value and fruit production (Figure 4) [6]. However, it was also possible that one or more of these lines arose independently, generating a mutation in the same nucleotide as s-classic. To address this question, we sequenced the coding region of all 22 lines and un-branched controls and found that all were identical except CC5721. Interestingly, this line carried four single-nucleotide polymorphisms (SNPs) that were shared with at least one un-branched variety, indicating that the s mutation in CC5721 may have arisen independently from a genetically distinct progenitor line (GenBank accessions FJ190665, FJ190666, and FJ190667). Two pieces of evidence lend support to this claim. Firstly, the four SNPs were distributed close (all within 1,000 bp) to the s lesion. Secondly, we sequenced a short segment of DNA from the tightly linked bacterial artificial chromosome (BAC) 298N3 and found that CC5721 had six SNPs and a 9-bp insertion-deletion (indel) that distinguished it from the other 21 domesticated types carrying s-classic (Figure 3b) (GenBank accessions FJ215691 and FJ215692). Still, in the absence of a geographic distribution of haplotypes, we cannot exclude a remote possibility that CC5721 arose as a result of an intragenic recombination between s-classic and an unbranched variety. Regardless, at least three independently arisen alleles of s (s-classic, s-multiflora, and Rose Quartz Multiflora) are responsible for a major portion of the diversity in tomato inflorescence architecture.
anantha Encodes an F-Box Ortholog of Arabidopsis UNUSUAL FLORAL ORGANS (UFO)

The similarity between the phenotypically strong allele s-multiflora and strong an mutants suggested a functional link in regulating an underlying inflorescence branching program (Figure 1). Furthermore, we created double mutant plants of weak an alleles and s and found they were phenotypically enhanced to resemble strong an (Figure S5). Interestingly, stronger phenotypes were observed for both inflorescence branching and floral identity. Specifically, we found that the sepal and carpelloid tissue of weak an mutants became much more meristematic with less organ identity (Figure S5B). In some double mutants, additional leaves formed in the inflorescence, resembling fa mutants (unpublished data). This suggests that S and AN have overlapping roles in inflorescence architecture as well as floral identity. We noted that an resembled a Lotus japonicus mutant called proliferating floral organs (pfo) (Figure 1e) [15]. PFO encodes an F-box protein orthologous to Antirrhinum FIMBRIATA (FIM) and Arabidopsis UNUSUAL FLORAL ORGANS (UFO) [16], and the tomato ortholog of this gene co-segregated with an. Six alleles had mutations in the coding region, revealing that an is mutated in the tomato ortholog of FIM/UFO (Figures 2C, 3E, and Figure S3A and S6) (GenBank accession FJ190668). The similar inflorescence and floral phenotypes found in an and fa mutants [17] may, therefore, stem from conserved functional associations of their gene products as described in Arabidopsis [18]. However, the relationship between S and AN was less clear, and their expression patterns were therefore explored.

Figure 1. Solanaceae Inflorescences and Mutant Phenotypes
(A) Pepper plant showing single-flower inflorescence and mature fruit (inset).
(B) Tomato plant and inflorescence (red ring) showing zigzag growth (lower inset) and maturing fruits (upper inset).
(C) Branched inflorescence of the species S. crispum.
(D) Mutant, highly branched inflorescence of s in a mixed genotype with the wild tomato species S. pennelli.
(E) Mutant inflorescence of a second allele, s-multiflora, having flowers (blue arrows) mixed with cauliflower-like tissue.
(F) Mutant, branched an-classic inflorescence with cauliflower-like tissue in place of flowers.
(G) A weaker an allele with sepal and carpelloid tissue.
(H) Mutant, branched fa inflorescence (dashed box) with leaves in place of flowers. Scale bars in (A, B, C, D, H), 5 cm; in insets (E, F, and G), 1 cm.
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S and AN Are Sequentially Expressed to Promote the Gradual Transition from Inflorescence to Flower Meristem

S was expressed to varying degrees in all tissues except roots, whereas most AN expression was restricted to floral buds, indicating a primary function in inflorescence and flower development. FA accumulated predominantly in shoot apices (Figure 5A)[9]. We explored further the expression of S and AN using in situ hybridization, which revealed temporally distinct patterns during inflorescence development. S was expressed in a wedge shape radiating outward from 2–3 cells from the center of immature SIMs (Figure 5B and 5C). This expression initiated shortly after lateral bulging of the SIM and was transient, because it disappeared before floral termination. AN expression initiated in incipient FMs shortly after down-regulation of S. AN expression was less intense than S, and was limited to the upper layers of the rapidly maturing SIM (Figure 5D and 5E). Both genes were reactivated...
Figure 2. Early Branching Patterns of Normal and Mutant Inflorescences

Scanning electron micrographs and schematics of inflorescence development. Schematics reflect sequential inflorescence sympodial units (ISU) each composed of a SIM branch (colored line with arrow) that terminates with a flower (FM, colored oval). Colored circles in micrographs reflect corresponding structures in schematics.

(A) Two stages of sympodial inflorescence development and mature zigzag inflorescence.

(B) s inflorescences develop extra SIMs due to mutations in the ortholog of WOX9 (red rectangle = homeodomain; mutations marked by red arrows = s-classic and s-multiflora). Additional SIMs (colored circles) eventually form flowers. Black asterisks reflect asymmetrical development of meristem branches (black arrows in schematics).

(C) Strong alleles of an, defective in the tomato ortholog of UFO, produce extra SIMs instead of flowers (blue rectangle = F-box domain; mutations marked by red arrows). Same color dots and lines reflect SIMs of a similar stage that become branches of the mature inflorescence (see Figure S1 for more details).

(D) S. crispum inflorescences showing an s-like SIM branching pattern (colored dots/asterisks reflect interpretation of sequential SIM production similar to the convention in (B)). The youngest inflorescence (left) has already produced three SIMs from the leading SIM (red dot), and each of these elaborates further (middle). A later stage inflorescence (right) shows more than 50 maturing flowers. As seen in s, the number and position of lateral SIMs derived from leading meristems varies between inflorescences, as does the position of differentiating flowers (see Figure S2 for more details). L = leaf; SYM = sympodial shoot meristem. Scale bars, 100 μm.

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in flower primordia in a ring of cells that marked a boundary domain, first between sepal and petal primordia and later between petals and stamens. These floral expression patterns are consistent with the failure of an mutants to initiate normal flowers, suggest a role for S in the flower, and likely explain the enhanced developmental and molecular phenotypes that s imposes on floral organ identity in weak alleles of an (Figure 3). Indeed, double mutants show little or no an expression similar to strong an mutants alone (Figure S5D).

The expression pattern of S suggested that it functioned
early in SIM maturation to promote the transition to FM, whereas AN operated soon after to provide early FM identity.

To test these hypotheses, we examined the expression of S and AN in s, an, and fa mutants. S was expressed in all mutant backgrounds, and, as in wild-type, was detected in younger lateral SIMs of an inflorescences (Figure 5F and 5G). This indicates that an meristems still reach a pre-floral SIM state. AN expression, on the other hand, was undetectable by RT-PCR (reverse-transcriptase PCR) in fa mutants, consistent with the proposal that FA functions upstream of AN [17](Figure 5F). Initial expression of AN in s mutants was delayed, and subsequently detected in only a small subset of SIMs compared to wild-type. In those meristems expressing AN, the signal was deeper and more intense than normal (Figure 5H). In situ hybridization from older inflorescences revealed some meristems lacking S and AN activity altogether, which we verified by whole-mount in situ hybridization (Figure 5H and unpublished data). This indicates that different meristems are at different phases of ISU maturation, and may also reflect the frequent observation of modified leaves or bracts in older an inflorescences if some meristems retain a more vegetative state. Taken together, these expression patterns support a mechanism where S and AN promote successive stages in the progression of an inflorescence meristem to floral specification through sequential transient activities that gradually promote maturation of SIMs (expressing S) to early FMs (expressing AN) (Figure 5I). Loss of either gene provides SIMs with an extended period of indeterminacy that facilitates ISU elaboration according to an underlying program of sympodial growth (Figure 5J).

Figure 4. Inflorescence Variation in Domesticated Tomatoes Is Due To Independently Arisen Alleles of s

The s-classic allele was first described 100 years ago as a highly branched variety called “Wonder of Italy,” and garden varieties resembling s remain popular for their aesthetic value and prolific fruit production [38]. Six thousand domesticated varieties were screened for inflorescence variation and 23 lines exhibited highly compound inflorescences. Among the 23 lines, at least 15 represented distinct genetic backgrounds based on differences in fruit size, shape, color, and quantitative variation in branch number.

(A) Phenotypic variation from three distinct varieties is shown. Core Collection line 2064 (CC2064) was extremely compound as a result of more than 200 branching events, whereas CC944 and CC3381 branched less often, and CC3381 also developed leaves within the inflorescence. (B) Variation in fruit size, shape, and color highlighting the different genetic backgrounds of the varieties with compound inflorescences. Varieties with names are indicated. (C) Cleaved amplified polymorphic sequence (CAPS) PCR genotyping assay showing that all except one of 23 varieties with compound inflorescences carry the s-classic allele. CC5721 (white asterisk), which carries the identical lesion as s-classic, arose independently from a distinct progenitor line (see text for details). Controls were varieties with weak (5–10 branching events) or no branching. Rose Quartz Multiflora was confirmed by complementation test to be an allele of s, and arose independently as a result of a genomic rearrangement (Figure S3). Scale bar in (A), 1 cm.

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Figure 5. Expression Patterns of Three Inflorescence Architecture Genes

(A) RT-PCR of S, AN, and FA transcripts in normal tissues.
(B–E) Detection of S and AN by in situ hybridization. Upper right denotes probe; lower left, genotype.
(B) Longitudinal section of a normal inflorescence showing S expression in an immature SIM (lower red arrow), but not in a more advanced ISU (upper blue arrow). Weak expression is observed between sepal and petal primordia in flowers (black arrows).
(C) Close-up of similarly staged section from (B).
(D) Longitudinal section showing AN expression in an incipient FM in the upper ISU (blue arrow). Expression is absent in the lower immature SIM (red arrow). AN is also expressed between sepal and petal primordial.
(E) Close-up of a similarly staged section.
(F) RT-PCR of S, AN, and FA in normal and mutant inflorescences (IF).
(G) Expression of S in an mutants marking SIMs that remain in a pre-floral state.
(H) In situ hybridization with whole-mounted tissue from an s mutant; AN expression in an advanced SIM (blue arrow), but not in a less mature SIM below (red arrow), matching a similarly staged section (inset).
(I) Sequential transient expression of S and AN. The first SIM (SIM1) expresses S (S1) and initiates the first phase of the maturation of ISUs. This expression is transient as it turns off prior to activation of AN (AN1) during the second phase of maturation, which occurs in the same ISU (ISU-1). A newly formed SIM (SIM2) emerges laterally marked by a new round of S expression (S2), which begins maturation of ISU-2, and this process reiterates to produce a multi-flower inflorescence. (J) Schematic for temporal development (color gradient in bar) over time (position in bar) of a normal (WT) ISU. The SIM (yellow) is short-lived and transitions rapidly (orange) to a FM (red) via activity of S (black line above yellow) followed by a short period of expression from AN (black line above orange). Mutant ISUs of s temporarily stall as SIMs (extended yellow bar) allowing extra SIMs to develop before terminating in FMs. an mutants remain in a pre-floral state (extended yellow bar with S expression) enabling SIMs to elaborate indefinitely. Se = sepals; St = stamens.
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Furthermore, the observation that S expression is maintained in an mutants and vice-versa, and that their expression is restricted to temporally distinct domains, supports the notion that these genes have separate but overlapping functions in the maturation of individual ISUs, consistent with the enhancement of weak an alleles by s (Figure S5).

**A Branched Inflorescence Is Based on the Gradual Transition of an Inflorescence Meristem to a Floral Meristem**

The expression patterns of S and AN along with their mutant phenotypes lead to a model in which temporal differences in the maturation of a SIM to an FM can regulate the duration of sympodial inflorescence branching. In other words, a slower transition enables more inflorescence branching and vice-versa. This suggests that the SIM phase and the early FM phase of a single flower can each provide a developmental window in which a compound inflorescence can form. We tested this hypothesis genetically by taking advantage of mutants of single flower truss (the tomato ortholog of FT, which is a major component of florigen), whose inflorescences are indeterminate vegetative shoots with single flowers separated in space by leaves [19] (Figure 6A). In sftan double mutants, we observed that individual flowers became branched inflorescences, though less so than in an mutants alone (2–4 versus 20–25 branches at the same age, Figures 1F, 6B, and 6C). By contrast, branching in sft:s double mutants resulted in elaboration of the vegetative inflorescence, but normal flowers still formed (Figure S7). Taken together, these results support the proposal that S acts earlier within a single inflorescence meristem to regulate sympodial branching, whereas AN acts later as FM identity is reached.

Our model suggests that Solanaceae inflorescences with only single flowers may result from rapid termination of the FM and hence elimination of the SIM stage, but that single flower species can still produce branched inflorescences. We
addressed this by mutagenizing pepper (C. anuum), which identified one mutant (called Ca-an) that produced an indeterminate shoot instead of a flower. This structure lacked petals and stamens and branched more extensively in a mixed genetic background, resembling tomato an mutants (Figure 6E and Figure S8). We sequenced pepper AN from Ca-an and found a missense mutation from the wild-type progenitor sequence causing a nucleotide change just prior to one of our tomato an alleles (an-e1444) that co-segregated with the mutant phenotype (Figure S6), indicating that Ca-an is mutated in the pepper ortholog of FIM/UFO (GenBank accession EF1906699). Like tomato, Ca-AN was expressed in a ring of cells flanking developing petals and stamens (Figure S8). Interestingly, Ca-AN could not be detected in an earlier inflorescence meristem, which lends support to the idea that pepper has a short SIM phase and progresses rapidly to floral termination. Yet, Ca-an mutants revealed a latent potential to branch, indicating that Solanaceae AN shares a conserved role in promoting FM determinacy with its orthologs in other species [15,16,20,21]. Of all other known UFO mutants, the pfo mutant from L. japonicus is most similar to Ca-an, with a compact branched structure described as a reiteration of sepal and FM organs. Normal L. japonicus produces pairs of flowers in the axils of leaves, and so loss of UFO function provides an extended period of indeterminacy to each pair of inflorescence meristems. By contrast, the stp mutant of pea generates similar organ defects but produces secondary FMs within the primary flower. Thus, UFO has a highly conserved role in floral identity, but its control of inflorescence branching is more species-specific and likely reflects differences in mechanisms of inflorescence meristem initiation. Notably, branching of tomato an mutants was more extreme than in Ca-an mutants (Figures 1 and 6). This indicates that underlying the tomato SIM phase is a program promoting branching and that the foundation for more complex branching is an inflorescence composed of reiterated SIMs. These data suggest that highly branched species like S. crispatum evolved from an ancestral form that resembled tomato, as opposed to pepper (Figure S2).

Discussion

Our results reveal a genetic foundation for the Solanaceae inflorescence and provide evidence for a possible mechanism that modulates simple and complex inflorescence structures known as “cymes” [1,2]. While the generation of a cyme inflorescence through sympodial growth is likely a complex process involving many unknown genetic and environmental factors, we provide a major advance in understanding how cymes may be modified into more complex structures based on elaboration of the ubiquitous ISU shoot system (Figure 2) [5]. This mechanism uses conserved machinery (AN/UFO and FA/LFY) that regulates inflorescence and flower development in other species [15,16,20–26]. Interestingly, the effects of UFO on inflorescence architecture vary considerably, ranging from infrequent replacement of single flowers with secondary inflorescence shoots in Arabidopsis ufo mutants [27], to the production of ectopic flowers in the inflorescences of pea stp mutants [20], to the large mass of inflorescence/floral tissue in pfo mutants of L. japonicus [15], and as shown here, the an mutant of tomato and pepper. Furthermore, we describe S/WOX9 as a novel component in the control of inflorescence architecture—a role that was not detected for its Arabidopsis ortholog. We also find that the tomato ortholog of TERMINAL FLOWER1 (TFL1) called SELF PRUNING (SP), which has a major effect on Arabidopsis inflorescences [2], is neutral on sympodial inflorescence branching in normal tomato inflorescences (unpublished data), and exhibits indirect effects on s inflorescence branching (Table S1). These differences may originate from the evolution of distinct growth habits. Branching complexity in sympodial species relies on termination of inflorescence meristems through the transition of a SIM to an FM. We suggest that a transient expression of S followed by AN was co-opted in Solanaceae sympodial development to boost two phases of sympodial meristem growth in this specialized shoot, both of which can potentiate branching (Figure 3I). In monopodial dicot species such as Arabidopsis or Antirrhinum, the inflorescence meristem produces no comparable SIMs, being indeterminate and generating lateral single flowers. This indeterminacy may explain why WOX9, by itself, is dispensable for inflorescence development [28,29]. Indeed, inflorescence ramification in monopodial dicot plants is more often stimulated through identity change [16,30,31], which could also explain some of the branching effects observed in Ca-an (Figure 6).

Thus, while the evolutionary diversification of plant inflorescence architecture is united under a common developmental theme [2], plants with different growth habits use related as well as distinct developmental modules to regulate branching [32]. We propose that Solanaceae inflorescence variation is based on controlling sympodial branching through temporal changes in the acquisition of floral fate, which is most flexible within the SIM phase. Short delays in the activation of genes like S (or as-yet-undiscovered other genes in the S pathway) followed by an abrupt switch to floral termination may explain the evolution and quantitative variation of compound inflorescences in the genus Solanum (Figure S9), as well as in other sympodial species, like trees [5]. Such a mechanism would provide a flexible way to guarantee the production and simultaneous maturation of large numbers of flowers, thereby ensuring a crucial aspect of reproductive success and perhaps providing a new tool for the manipulation of crop yields.

Materials and Methods

Plant material and gene cloning. Classic alleles of s (s-classic LA3094), an (LA0536), and fa (LA0854), and those of representative wild tomato species were gifts from the C. M. Rick Center (Davis, California; http://tgrc.ucdavis.edu). An additional allele of s (LA0560; s-multiflora, C. M Rick Center) was verified by complementation test. A third s allele and six additional an alleles were identified as inflorescence mutants in a screen of a tomato mutant library [8]. Wild tomato species were gifts from the C. M. Rick Center. Wild tomato species, such as S. lycopersicoides, can be difficult to grow and maintain until flowering and only two representative plants were available for phenotypic analyses, but inflorescence complexity within each plant was uniform throughout. More distantly related Solanum species were gifts from the Botanical and Experimental Garden at Nijmegen, The Netherlands. Up to three representative plants were used for phenotypic analyses. The ~6,000 domesticated tomato varieties were collected from various public and private germplasm sources. All plants were grown in greenhouses under natural light or in agricultural field conditions in Israel using standard irrigation and fertilization regimes.

Compound inflorescence (s). The s mutant was originally mapped on the long arm of chromosome 2 and verified using 29 mutants selected from an F2 population derived from a cross with the wild species S. pimpinellifolium (LA1589). This positioned s in the region overlapping introgression lines II.2–3II.4 on the tomato introgress...
sion line map [33]. A larger mapping population was generated by crossing s-5568 with the wild tomato species S. pennellii (LA0716). F1 hybrid plants were self-fertilized to produce a mapping population of 3,000 F2 plants. Five hundred individual s mutant plants were scored with CAPS-PCR markers from the most current tomato genetic map (Solanaceae Genomics Network at http://www.sgn.cornell.edu), focusing on restriction site differences IL2–3/2–4. Additional s mutant markers supported the previously tightly linked CCR locus were provided by K. Manning [34]. Marker density was improved using conserved synteny identified between seven markers in a 15-CM window in tomato and a 500-kb segment of Arabidopsis chromosome 1 (marker information available upon request). Two co-segregating markers (0 recombinant chromosomes out of 1,000 gametes) were used to isolate a BAC from a S. lycopersicum HindIII library kindly provided by J. J. Giovannoni and J. Van Eck at Cornell University (Ithaca, New York). DNA fragments from three independent restriction enzyme digestion sing a single BAC clone were used to produce a BAC library. A tomato TOPO TA library was constructed through Epicentre Biotechnologies using TriReagent (Sigma-Genosys). DNA from the mutant was isolated and sequenced using 454 shotgun sequencing. Fragments containing genes were annotated using BLASTX against the Arabidopsis protein database and used to search other genomes for additional synteny. Sequences from four tightly linked markers (Figure 3) were used in a BLASTN or TBLASTX search against genomes of P. hybrida, M. truncatula, and V. vinifera. Genes in syntic regions ranging from 110–140 kb were aligned manually and searched for candidate genes, which identified the Apetala2-2 (AP2) and Wuschel-homebox (WOX) transcript typing factors. A tomato TOPO TA library was constructed by degenerate PCR based on conserved regions in the WOX from these three species and an EST from Petunia hybridia (accession number EB174885). Transcript ends were determined by rapid amplification of cDNA ends (RACE) (Sambrook) using total RNA isolated from young inflorescences with TriReagent (Sigma-Genosys). DNA from s-like varieties with compound inflorescences from the core collection was PCR amplified with gene-specific primers and used in a CAPS-PCR assay diagnostic of s-classic.

The expressivity of the s phenotype is affected by genetic background, which became evident when phenotyping 22 domesticated varieties each carrying a s-classic allele, but varying in many phenotypic characters, including branching. Modifiers are responsible for these differences, which may or may not have a functional relationship to S. Furthermore, it is well-documented that sympodial shoots in tomato is highly sensitive to light intensity, which could also contribute to quantitative variation between accessions. On occasion, modestly branched accessions were observed that produced only 2–4 additional branches compared to normal, which, if not allelic to s, could potentially modify (enhance) the s phenotype. Yet, the majority of extreme branching variation was due solely to changes in S function, indicated by normal segregation of families segregating for each s allele in a common genetic background (cv. S82). Thus, differences in phenotypic strength, as seen in s-multiflora, result from modifier loci, but these are much weaker in their effects compared to s mutations.

**an** (anther)
The anther mutant was originally mapped to the long arm of chromosome 2, and subsequently positioned in the region overlapping IL2–3/2–4–5. The phenotype of the pfo mutant in L. japonicus resembled weak alleles of an and led us to search for the tomato ortholog of FIMUPO. A single EST (SGN-U341425) with homology to FIMUPO was used to generate a CAPS-PCR marker that mapped to the same region as an (http://www.sgn.cornell.edu). DNA from six EMS alleles was amplified using gene-specific primers and sequenced directly, which identified five independent mutations. The central portion of coding sequence of the an-classic allele could not be amplified, suggesting a structural change or large deletion (unpublished data). This rearrangement in the an-classic allele was verified using DNA Southern blot hybridizations (Figure S3) according to established protocols.

**Pepper (Capsicum annuum)** and the pepper anantha mutant (Ca-an) An EMS mutation of the pepper variety Maor was performed according to a protocol for tomato seeds [8]. Among 1,500 M2 families of pepper, one inflorescence mutant was identified based on phenotypic similarity to weak alleles of tomato an This mutant was first mapped by restriction fragment length polymorphism (RFLP) analysis to a region of chromosome 2 in pepper that is syntenic with tomato chromosome 2 where anantha was positioned (RFLP) analysis to a region of chromosome 2 in pepper that is syntenic with tomato chromosome 2 where anantha was positioned. DNA from the mutant was isolated and sequenced using primers designed from the tomato gene. Co-segregation of the mutation with the pepper an phenotype was verified in an F2 population of approximately 100 plants. Additional markers syntenic with pepper an were not in the region.

**Phenotypic and expression analyses.** Developmental and morphological responses to s mutations in double mutants were performed on alleles originating from the tomato cultivar M82. M82 lines were either mutant or wild type for the gene SELF PRUNING (SP), which had only modest effects on inflorescence phenotypes in s or an that could be attributed to changes in the length of sympodial units—a phenotype regulated by SP. Single and double mutants were first mapped by restriction fragment length polymorphism (RFLP) analysis to a region of chromosome 2 in pepper that is syntenic with tomato chromosome 2 where anantha was positioned (RFLP) analysis to a region of chromosome 2 in pepper that is syntenic with tomato chromosome 2 where anantha was positioned (Figure S3) accord-ing to established protocols.

**Supporting Information**

**Figure S1.** Temporal Progression of Early Branching Patterns in Normal and Mutant Inflorescences

Scanning electron micrographs of inflorescence development and corresponding schematics are shown. Colored lines and ovals in schematics reflect individual inflorescence sympodial units (ISUs) composed of a SIM branch that terminates in a flower (FM). Identically colored circles in micrographs reflect ISUs generated before floral termination of each leading SIM (colored circles). Flowers that form vary in number and position between inflorescences. Black asterisks (black lines in schematics) reflect asymmetrical development of additional meristem branches. Despite this asymmetry, relatively uniform branching patterns emerge in mature inflorescences (C–F).

**Figure S2.** Temporal Progression of Early Inflorescence Branching Patterns in the Wild Species S. cristatum

Scanning electron micrographs (numbered 1–8) present a developmental range of individual inflorescences from the transition to flowering (1) to multi-flower differentiation (7,8). Colored circles in micrographs reflect one possible interpretation of the sequential development of SIMs and this elaboration was also asynchronous (black asterisks). As well, the position of inclusions varies between inflorescences as seen for s in Fig. S1. Last stage inflorescences were too complex to be marked. At maturity, S. cristatum branched an average of 25 times and produced more than 100 flowers per inflorescence.
(A) Young branching inflorescence of a weak allele (an-c1436) having sepal and carpeloid tissue, but lacking petals and stamens. (B) Double mutant of an-c1465 with s-n5568 showing an enhanced inflorescence phenotype having stronger floral organ defects resembling strong alleles of an, like an-c1436 shown in (C). (D) RT-PCR of AN expression on single and double mutants. The weak allele an-c1436 shows a modest reduction in AN expression relative to WT inflorescences. The strong allele an-c1456 has little or no AN expression at all. Like a similar loss of expression is recapitulated in the enhanced double mutants s-n5568/an-c1436. This suggests either that S has a transcriptional regulatory role on AN or that the double mutant arrests at a developmental stage lacking early FM identity, and therefore does not express AN at substantial levels.

Found at doi:10.1371/journal.pbio.0060288.sg004 (1.64 MB JPG).

Figure S3. Genomic Rearrangements of Alleles of s and an

(A) DNA Southern blot showing genomic changes in an-c1436 (LA0530). Genomic DNA from wild type (WT), a mix of WT and heterozygous (HET), and mutant (an) plants from a segregating family was digested with restriction enzyme and probed with the full length AN gene. Band shifts were observed in an, but not in WT alone and WT/HET samples showed heterozygosity. One explanation for the an-c1436 allele is a transposon insertion within the gene, which would explain the increase in size of the mutant band. Consistent with this idea, we were unable to PCR amplify the central portion of the gene (unpublished data), indicating a chromosomal change in the coding sequence. However, other types of rearrangements could also explain this result, which we did not explore.

(B) DNA Southern blot showing genomic changes in s-n5568 and Rose Quartz Mutiflora (RQM). For both mutants, we were unable to find mutations by sequence analysis, and we were unable to PCR amplify the 3’ end of s in RQM mutants, suggesting genomic changes for both alleles. Genomic DNA from wild-type (WT), domesticated cultivar, M82 and mutant plants of s-n5568 (M82 background) and RQM (unknown background) was digested with five restriction enzymes and hybridized with a probe corresponding to the 2’ portion of s gene. For s, a lower band shift (red arrows) was observed for two enzymes, suggesting a large deletion. s-n5568 was produced in a fast neutron mutagenesis, which is consistent with this idea; however, the deletion would have to reside upstream or downstream of the coding sequence, because we were still able to amplify the 3’ end of the gene by PCR, and we still detect transscripts by RT-PCR and in situ hybridization (unpublished data). Three enzymes revealed band shifts (blue arrows) in RQM relative to WT. Such a high frequency of intraspecific polymorphisms by DNA blot is rare in domesticated tomato varieties [37], suggesting these changes are associated with the mutant phenotype. A number of rearrangements are possible, which we did not explore further.

Found at doi:10.1371/journal.pbio.0060288.sg003 (1.03 MB JPG).

Figure S4. Multiple Alignment of S with Highly Related WOX9 Proteins

Highly conserved amino acids are shaded in black, and different amino acids of the same group are shaded in gray. The consensus sequence is shown below the alignment. Dashes denote gaps that were introduced to optimize the alignment. The homodomain is boxed. The mutations for two missense alleles of s (s-c1465 and s-multiflora) causing amino acid changes of invariant residues in the homodomain are indicated above the alignment in red font. Sl-S: Solanum lycopersicum S; Ph-WOX9A: Petunia x hybrida WOX9A (GenBank accession number EB174497); Ph-WOX9B: Petunia x hybrida WOX9B (accession number EB174498); Pt-WOX9A: Populus trichocarpa (accession number AF004843); Pt-WOX9B: Populus trichocarpa (genome protein ID: 555728); Mt-WOX9: Medicago truncatula (accession number ABN09121); Vv-WOX9: Vitis vinifera (accession number CA066373); At-WOX9-STIP: Arabidopsis thaliana Stamiply-like (accession number NP_180944); At-WOX9-STIPL: Arabidopsis thaliana Stamiply-like (accession number NP_199410).

Found at doi:10.1371/journal.pbio.0060288.sg004 (2.73 MB JPG).

Figure S5. Enhanced Inflorescence and Floral Phenotypes in Double Mutants between s and Weak Alleles of an

(A) Young branching inflorescence of a weak an allele (an-c1435) having sepal and carpeloid tissue, but lacking petals and stamens. (B) Double mutant of an-c1465 with s-n5568 showing an enhanced inflorescence phenotype having stronger floral organ defects resembling strong alleles of an, like an-c1436 shown in (C).

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Figure S6. Multiple Alignment of S with Highly Related F-Box Proteins

Highly conserved amino acids are shaded in black, and different amino acids of the same group are shaded in gray. The consensus sequence is shown below the alignment. Dashes denote gaps that were introduced to optimize the alignment. The F-box domain is boxed.

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Figure S7. Inflorescence Branching Phenotypes in Double Mutants of s and an

Double mutants between s and an convert the single indeterminate vegetative inflorescence shoot of s mutants to a highly branched vegetative inflorescence (red ring, white arrow) with dispersed single flowers (yellow arrowheads). SIM elaboration from a young developmental stage (inset) shows branches composed of leaflets and floral buds (red arrowheads) developing behind a single terminal flower (yellow arrowheads). These are the vegetative inflorescence branches that contribute to the architecture of mature double mutant inflorescences.

Scale bars, 10 cm plant; 100 µm, inset.

Found at doi:10.1371/journal.pbio.0060288.sg008 (3.46 MB JPG).

Figure S8. Early Development and AN Expression in Inflorescences of Normal Pepper and Ca-an Mutants

(A–D) Scanning electron micrographs showing two sympodial shoots (SYM) and distinct inflorescence stages from normal (A and C) and Ca-an mutants (B and D). Normal pepper produces a single floral meristem (FM, red dot) flanked by two SYMs, which are composed of two leaves and a single flower that terminates rapidly. Sympodial shoots arise reiteratively in the axils of each sympodial leaf and are released from apical dominance asymmetrically after floral termination, which is reflected in the slightly different developmental stages. Ca-an mutants produce an indeterminate shoot (asterisk) that repeatedly gives off lateral organs, which are, perhaps, modified sepalas. Branching is infrequent at this early stage, and occurs more often in mature inflorescences or in the presence of modifiers, as shown in Figure 6F.

(E and F) Detection of pepper Ca-AN expression by in situ hybridization. (E) Longitudinal section from a young inflorescence showing expression in the developing FM interior to sepal primordia (black arrows), but not in flanking SYMs (red arrows).

(F) A later stage inflorescence with early stage FMs (left and right). The earliest expression of Ca-AN is observed in a ring (black arrows) between sepalas (Se) and incipient petals. Se= stamen; L= leaf; SYM=symodial meristem; FM= floral meristem. Scale bars, 100 µm.

Found at doi:10.1371/journal.pbio.0060288.sg009 (674 KB JPG).

Figure S9. Inflorescence Variation in the Genus Solanum

Quantification of inflorescence branching events from five species in the genus Solanum revealing that inflorescence branching varies widely between species and may have evolved multiple times. Numbers in parentheses indicate average number of branching events in each inflorescence.

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Table S1. The Tomato Ortholog of TFL1, called SP, Does Not Directly Influence Inflorescence Architecture

Comparison of inflorescence branching events in three consecutive inflorescences from s mutant plants carrying a functional (SP+) or mutant (sp−) copy of SP, the tomato ortholog of TFL1. The first inflorescence after the transition to flowering (primary IF) showed a modest decrease in branching in sp− double mutants (highlighted in gray). Interestingly, this effect was reversed in the following inflorescences of the first IF (SYM IF) and in SYMs of the following IF, suggesting that sp− plants, which showed more branching events than in s mutants alone (highlighted in gray). These effects are primarily indirect effects of sp− mutants, whose primary change is on sympodial unit length (i.e., the number of leaves between sympodial units), which decreases progressively as sp− plants mature [10]. This allows the initiation of both the first and second SYM IF in sp− to occur earlier and, therefore, undergo more branching events compared to correspondingly younger inflorescences in s mutants.
alone. Eventually these younger inflorescences underwent a similar number of branching events, although more variation was introduced as inflorescences aged (unpublished data).

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Author contributions

ZBL, JPA, YE, I. Paran, and DZ designed research ZBL, OC, JPA, MA, and I. Pekker performed research. ZBL, JPA, YE, I. Paran, and DZ analyzed the data. ZBL, YE, and DZ wrote the paper.

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Competing interests

The authors have declared that no competing interests exist.

References

1. Rickert HW (1944) The classification of inflorescences. Bot Rev 10: 187–231.
2. Prusinkiewicz P, Erasmus Y, Lane B, Harder LD, Coen E (2007) Evolution and development of inflorescence architectures. Science 316: 1452–1456.
3. Darlington CJ (1948) The morphology of flowering plants in reproductiven Bereich. Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin 6: 1–183.
4. Child A (1979) A review of branching patterns in the Solanaceae. In: Garrett FG, Lester RN, Skelding AD, editors. The biology and taxonomy of the Solanaceae. London: Academic Press. pp. 345–356.
5. Halle F, Oldeman RAA, Tomlinson PB (1978) Vergleichende Betrachtungen über die Entwicklung der Infloreszenz bei Lycopersicum esculentum Mill. und bei einer Rotenmu- tanze. Der Zuchter 21: 89–95.
6. Menda N, Semel Y, Peled D, Eshed Y, Zamir D (2004) In silico screening of a saturated mutation library of tomato. Plant J 38: 861–872.
7. Molinero-Rosales N, Jamilena M, Zurita S, Gomez P, Capel J, et al. (1999) FAM14A, the tomato orthologue of FLORICAULA and LEAFY controls flowering time and floral meristem identity. Plant J 20: 685–693.
8. Pruneli L, Carmel-Goren L, Haveven D, Güttinger T, Alvarez J, et al. (1998) The SELF-PRUNING gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of CEN and TFL1. Development 125: 1979–1989.
9. Schmitz G, Theres K (1999) Genetic control of branching in Arabidopsis and tomato.Curr Opin Plant Biol 2: 51–55.
10. Haeker A, Gross-Hardt R, Geiger B, Sarkar A, Breuninger H, et al. (2004) Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in Arabidopsis thaliana. Development 131: 657–668.
11. Wu X, Chory J, Weigel D (2007) Combinations of WOX activities regulate tissue proliferation during Arabidopsis embryonic development. Dev Biol 309: 306–316.
12. Tanksley SD (2004) The genetic, developmental, and molecular bases of fruit size and shape variation in tomato. Proc Natl Acad Sci U S A 101: 9398–9403.
13. Van Deynze A, Stoffel K, Buell CR, Kozik A, Liu J, et al. (2007) Diversity in conserved genes in tomato. BMC Genomics 8: 465.
14. Male CJ (1999) 100 Heirloom tomatoes for the American garden. New York: McCall’s Cooking Light.
15. Hejatko J, Blilou I, Brewer PB, Friml J, Scheres B, et al. (2006) In situ hybridization technique for mRNA detection in whole mount Arabidopsis samples. Nat Protoc 1: 1939–1946.
16. Van Deynze A, Stoffel K, Buell CR, Kozik A, Liu J, et al. (2007) Diversity in conserved genes in tomato. BMC Genomics 8: 465.
17. Fim and UFO, is required for normal development of flowers, inflorescences, and leaves. Plant Cell 13: 31–46.
18. Chae E, Tan QK, Hill TA, Irish VF (2008) An Arabidopsis F-box protein acts as a transcriptional co-factor to regulate floral development. Development 135: 1235–1245.
19. Lifschitz E, Eviatar T, Rozman A, Shalit A, Goldshmidt A, et al. (2006) The fruit size and shape variation in tomato. Plant Cell 16 Suppl: S181–189.
20. Allen KD, Sussex IM (1996) Falsiflora and anantha control early stages of flower development in foxtail millet. Proc Natl Acad Sci U S A 93: 1245–1249.
21. Tanksley SD (2004) The genetic, developmental, and molecular bases of fruit size and shape variation in tomato. Plant Cell 16 Suppl: S181–189.
22. Lee I, Wolfe DS, Nilsson O, Weigel D (1997) A LEAFY co-regulator encoded by UNUSUAL FLORAL ORGANS. Curr Biol 7: 95–104.
23. Rebocho AB, Bliek M, Kusters E, Procissi A, Mariková M, Matthes P, et al. (1997) UNIFOLIATA regulates leaf and flower morphogenesis in pea. Curr Biol 7: 581–587.
24. Angenent GC, Stuurman J, Snowden KC, Koes R (2005) Use of Petunia to unravel plant meristem functioning. Trends Plant Sci 10: 245–250.
25. Wang H, Chen J, Wen J, Tadese M, Li G, et al. (2008) Control of compound leaf development by FLOWERING LOCUS T and LEAFY in Petunia. Planta 223: 581–587.
26. Hefworth SR, Klenz JE, Haughn GW (2006) UFO in the Arabidopsis inflorescence apex is required for floral-meristem identity and bract suppression. Planta 223: 769–778.
27. PloS Biology | www.plosbiology.org November 2008 | Volume 6 | Issue 11 | e2882435
28. Pruneli L, Carmel-Goren L, Haveven D, Güttinger T, Alvarez J, et al. (1998) The SELF-PRUNING gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of CEN and TFL1. Development 125: 1979–1989.
29. Schmitz G, Theres K (1999) Genetic control of branching in Arabidopsis and tomato. Curr Opin Plant Biol 2: 51–55.
30. Haeker A, Gross-Hardt R, Geiger B, Sarkar A, Breuninger H, et al. (2004) Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in Arabidopsis thaliana. Development 131: 657–668.
31. Wu X, Chory J, Weigel D (2007) Combinations of WOX activities regulate tissue proliferation during Arabidopsis embryonic development. Dev Biol 309: 306–316.
32. Tanksley SD (2004) The genetic, developmental, and molecular bases of fruit size and shape variation in tomato. Plant Cell 16 Suppl: S181–189.
33. Zhang S, Polowick PL, Stiller J, Stougaard J, et al. (2003) Proliferating Floral Organs (Plo), a Lotus japonicus gene required for controlling flower development in Arabidopsis and Antirrhinum. Plant Cell 7: 1501–1510.
34. Allen KD, Sussex IM (1996) Falsiflora and anantha control early stages of floral meristem development in tomato (Lycopersicum esculentum, Mill.). Plant Cell 200: 254–264.
35. Chae E, Tan QK, Hill TA, Irish VF (2008) An Arabidopsis F-box protein acts as a transcriptional co-factor to regulate floral development. Development 135: 1235–1245.
36. Lifschitz E, Eviatar T, Rozman A, Shalit A, Goldshmidt A, et al. (2006) The fruit size and shape variation in tomato. Plant Cell 16 Suppl: S181–189.
37. Van Deynze A, Stoffel K, Buell CR, Kozik A, Liu J, et al. (2007) Diversity in conserved genes in tomato. BMC Genomics 8: 465.
38. Male CJ (1999) 100 Heirloom tomatoes for the American garden. New York: Workman Publishing Group.
39. Rebocho AB, Bliek M, Kusters E, Castel R, Procissi A, et al. (2006) Role of EVERGREEN in the development of the cymose petunia inflorescence. Dev Cell 15: 417–447.
40. Sover E, Rebocho AB, Bliek M, Kusters E, de Bruin RA, et al. (2008) Patterning of Inflorescences and Flowers by the F-Box Protein DOUBLE TOP and the LEAFY Homolog ABERRANT LEAF AND FLOWER of Petunia. Plant Cell 20: 2035–2048.

Note Added in Proof

While this article was under review, additional reports [39,40] have indicated that the orthologs of S and AN in Petunia hybrida (also a Solanaceae species), called EVERGREEN (EVG) and DOUBLE TOP (DOT), have similar functions within inflorescence meristems.