An integrated view of quantitative trait variation using tomato interspecific introgression lines

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Resolving natural phenotypic variation into genetic and molecular components is a major objective in biology. Over the past decade, tomato interspecific introgression lines (ILs), each carrying a single 'exotic' chromosome segment from a wild species, have exposed thousands of quantitative trait loci (QTL) affecting plant adaptation, morphology, yield, metabolism, and gene expression. QTL for fruit size and sugar composition were isolated by map-based cloning, while others were successfully implemented in marker-assisted breeding programs. More recently, integrating the multitude of IL-QTL into a single database has unraveled some unifying principles about the architecture of complex traits in plants.

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Introduction

The beauty of nature is founded in the networks of developmental programs that give rise to the variable shapes, forms, colors, and aromas of organs observed within and between species. Such complex variation is often manifested in a quantitative phenotypic range, as opposed to qualitative traits that fall into discrete 'Mendelian' categories [1,2]. The genetic variation underlying quantitative traits is notoriously difficult to dissect due to the segregation of numerous genes, or quantitative trait loci (QTL), each explaining a portion of the total variation, and whose expression is modified by interactions with other genes (epistasis) and the environment [3]. With the advent of DNA markers, it became possible to construct saturated genetic maps and to locate in linkage groups QTL for numerous phenotypes in plants, animals, and humans. More recently, the availability of whole genome sequences has assisted in enriching the marker repertoire and in developing new statistical methods for identifying QTL [4,5••]. Yet, still, a major bottleneck in the functional annotation of natural variation of both fundamental and applied importance is the availability of segregating genetic resources representing wide-diversity, and the sensitivity and scale of phenotyping platforms.

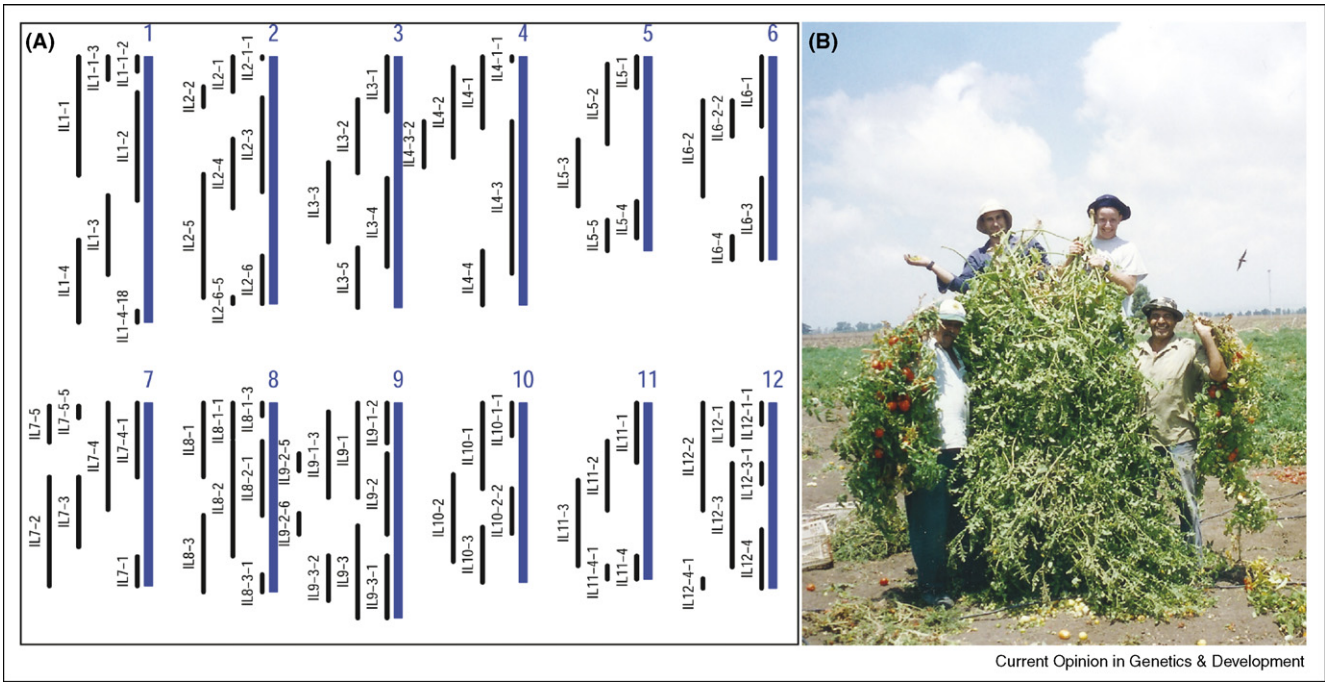
Plants are the founding models for quantitative trait genetics because they are particularly amenable to controlled population development and phenotyping, thereby permitting the use of DNA markers to map loci defining many aspects of multicellular growth and development. Among all model systems, the wild and domesticated species of the tomato clade in the family Solanaceae have pioneered novel population development that partition quantitative variation into Mendelian components [6]. This has facilitated the robust assessment of mean phenotypic values of QTL-containing chromosome segments — a challenging prerequisite for isolating complex trait genes [7,8]. Here, we review 15 years of research on the tomato introgression lines and how the integration of heterogeneous experiments into a unified database has provided a wider view of complex traits.

The tomato introgression lines

Traditionally, quantitative trait mapping studies in plants and animals involve whole genome segregating populations, but epistatic interactions in F2 or recombinant inbred lines (RILs), make it difficult to fully define and characterize individual loci. Introgression lines (ILs) are a set of nearly isogenic lines developed through a succession of backcrosses, where each line carries a single genetically defined chromosome segment from a divergent genome [9]. The term ILs, often used in plant biology, is synonymous with chromosome substitution strains (CSS) or 'congenics', which have been constructed for mice [10••,11,12]. A complete IL population reconstitutes the donor parent genome in overlapping chromosomal segments and is immortal since it can be maintained by self-pollination. Consequently, these populations are very effective in identifying and stabilizing QTL, because any phenotypic difference between an IL and the recurrent parent is attributed solely to one or more donor parent genes within the introgressed chromosomal segment.

The principles of the IL approach were first demonstrated in tomato. *Solanum lycopersicum* (domesticated tomato) is one of the 17 core species in the tomato clade, and is the most intensively studied of 3000 Solanaceae species, which include potato, pepper, and eggplant [13]. The small green-fruited desert species *Solanum pennellii* is a distant relative of *S. lycopersicum*, having evolved unique adaptations in terms of morphology, mating system,

Figure 1



The *Solanum pennellii* IL population. **(A)** Genome introgressions on the 12 tomato chromosomes of the 76 *S. pennellii* ILs, which are nearly is ogenic to each other and differ only for the marked introgressed chromosome segments. **(B)** Heterosis for plant biomass in the F1 hybrid of *S. pennellii* × *S. lycopersicum* (the middle plant) compared to the recurrent parent, M82 (far left and right plants). *S. pennellii*, while self-compatible in its native arid environment, does not set fruit in agricultural field conditions; however, it contributes QTL that significantly improve yield and other traits. The homozygous ILs show primarily lower yield than both parents owing to sterility, whereas certain IL hybrids show heterosis and increased yield. Interestingly, in many instances of crossing two ILs with similar QTL effects, double IL heterozygotes show lower magnitude than the sum of the effects of single heterozygotes, reflecting nonadditivity of canalized phenotypes [17].

chemistry (especially secondary compounds) and responses to biotic/abiotic stress. Despite these drastic ecological differences, *S. pennellii* is sexually compatible and produces fertile hybrids with *S. lycopersicum*, making it the founding donor parent of the first IL population used for interspecific QTL identification, cloning, and plant breeding. The ILs, representing whole-genome coverage of *S. pennellii* in overlapping segments in the genetic background of *S. lycopersicum* (cv. M82) presently consists of 76 genotypes (Figure 1). The *S. pennellii* ILs have been publicly available and have been phenotyped for hundreds of traits including repeated measurements of the same traits, thus allowing for the identification of 2795 QTL (Table 1). In the framework of a currently running EU project (EU-SOL), 500 sub-ILs are being produced to markedly improve mapping resolution.

Table 1

The prevalence and diversity of QTL identified by the *S. pennellii* ILs

QTL#	Trait#	Traits measured	Reference
36	6	Carotenoids content	McQuinn and Giovannoni, unpublished
18	2	Fruit phenotypes	White and Giovannoni, unpublished
6	1	Ascorbate (Vitamin C)	McQuinn and Giovannoni, unpublished
584	101	Primary metabolites	Y Semel , unpublished
889	74	Primary metabolites	Schauer *et al.* [29]
841	35	Morphology and yield	Semel *et al.* [18]
88	23	Volatile compounds	Tieman *et al.* [52]
20	3	Nutritional and antioxidant	Rousseaux *et al.* [53]
82	9	Sugars and acid content	Baxter *et al.* [54,55•]
81	9	Metabolites, brix and fruit weight	Causse *et al.* [56]
30	8	Leaf morphology	Holtan and Hake [57]
16	1	Intensity of red color of ripe fruit	Liu *et al.* [58]
104	6	Yield related traits	Eshed and Zamir [21]

Given the high phenotypic variability and the accumulation of years of communal data, the *S. pennellii* ILs have been ideally suited for exploring the genetic and molecular bases of environmental adaptation, reproductive biology, and many other topics [14,15•].

From QTL to gene

An important aspect of IL biology is the exposure of phenotypes not observed in the parents. This is referred to as transgressive segregation, which results from novel interactions between *S. pennellii* alleles and the independently evolved molecular networks of *S. lycopersicum* [16]. Although perhaps more common in F2 and RI populations, transgressive QTL in the *S. pennellii* ILs have been detected for both qualitative and quantitative traits including fruit shape, weight, yield, and color [17,18••]. For instance, mature fruits of *S. pennellii* are green, whereas most cultivated tomato varieties have red fruits. Interestingly, some ILs show novel fruit color variation, such as the dark orange fruits of IL6-3 (Introgression Line chromosome 6, segment 3) and IL12-2. The map-based cloning of the dominant orange gene of IL6-3 (designated *Beta*), which encoded a lycopene β-cyclase active in the carotenoid biosynthetic pathway [19], revealed that *S. pennelli Beta* is identical in coding sequence but carries six additional upstream sequence elements compared to the M82 allele. Consequently, lycopene β-cyclase is highly expressed from breaker to full fruit ripening in maturing fruits of IL6-3, giving rise to a higher content of β-carotene. In IL12-2 a single dominant gene, *Delta*, coding for lycopene epsilon-cyclase (*CrtL-e*), was shown to accumulate delta-carotene at the expense of lycopene, and, similar to *Beta*, RNA levels increased 30-fold over wild-type during fruit ripening [20]. These examples illustrate that the primary mechanism driving aberrant carotenoid accumulation, and perhaps other transgressive phenotypes, is novel epistatic transcriptional regulation of *S. pennellii* genes.

The aforementioned examples involve qualitative phenotypes, but early IL studies revealed that reproducible measurements and mapping could be achieved for quantitative traits, such as fruit weight and sugar content. Numerous loci with large and small effects on these two traits have been mapped in the ILs. IL2-5 carries at least three fruit weight QTL, the most significant of which is *fruit weight 2.2* (*fw2.2*) [21]. The generation of higher resolution near-isogenic lines showed that all large-fruited domesticated tomato varieties carry 'large-fruited' alleles of *fw2.2*, whereas 'small-fruited' wild alleles reliably reduce size by as much as 30% [7,22]. This germplasm enabled the cloning of *fw2.2*, coding for a protein with no known biological function. In developing fruits, *fw2.2* represses cell division, and different alleles exhibit heterochronic changes in gene expression accounting for their phenotypic effects. Consistent with this finding was that no changes in coding sequence could distinguish allelic functions, thereby pointing to *cis*-regulatory variation as the likely cause [23]. In this respect, the genetic basis of *fw2.2* resembles selection for promoter-based transcriptional variation at the *tb1* locus in maize — another domestication QTL [24••].

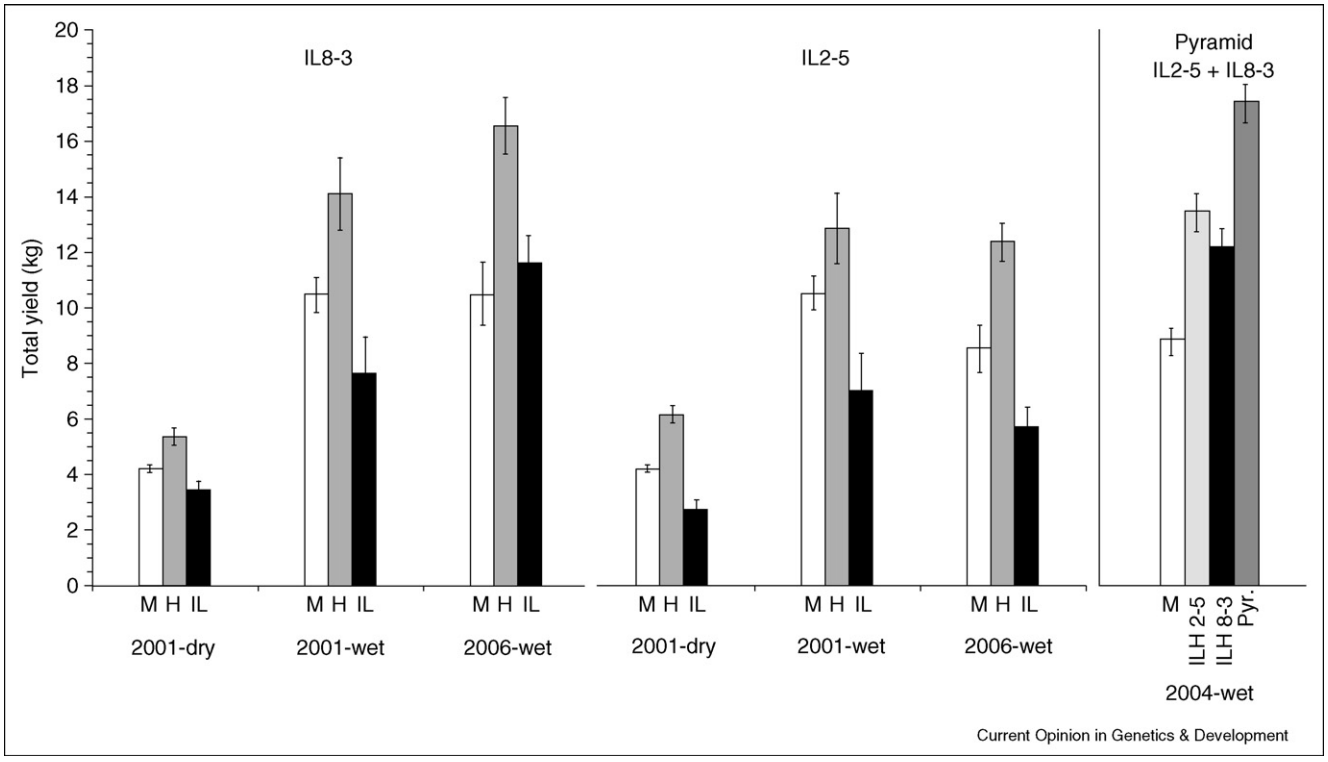
Concomitant with *fw2.2* was the cloning of a tomato sugar yield, or brix, QTL. *Brix9-2-5* increases sugars by as much as 25%, and using ultra-high resolution mapping, the molecular basis of this QTL was localized to a cell-wall invertase gene, *LIN5* [25]. The availability of IL populations for additional tomato species enabled a comparative association study between the nucleotide polymorphism and activity of *LIN5*, which pinpointed the effect of *Brix9-2-5* to a change in a conserved amino acid localized near the invertase active site responsible for sucrose recognition and binding [25]. Along with *fw2.2*, these examples of QTL gene isolation illustrated that continuous variation for quantitative phenotypes could be effectively partitioned into discrete molecular components through IL-based Mendelian segregation. Importantly, these QTL were the first among many showing that quantitative variation shares the same molecular features as laboratory-induced mutations, though an important distinction is that functional polymorphisms more often influence transcriptional regulation (*fw2.2*) as opposed to protein function (*Brix9-2-5*) [1].

Dissecting heterosis with the *S. pennellii* ILs

The immortality of the ILs has allowed multiple years of phenotyping over different environments, which has led to the reproducible identification of QTL for more integrated traits, such as yield and biomass. Perhaps not surprisingly, the vast majority of the ILs reduce yield in the homozygous state, which is a reflection of the evolution of interspecific reproductive barriers. However, when the ILs are hybridized to the recurrent M82 parent, a large number of yield-promoting heterotic QTL are revealed [18]. Heterosis, or hybrid vigor, occurs when hybrid offspring out-perform both parents for traits such as growth rate and yield, but its genetic and molecular bases remain obscure [26]. The *S. pennellii* ILs are proving to be a powerful tool to discover the loci that govern heterosis. In a large phenomic oriented field study, all ILs were hybridized with the recurrent parent M82 to test the effect of heterozygosity on 35 diverse phenotypes with the eventual identification of chromosomal segments that contribute to heterosis [18]. This phenomic analysis enabled the dissection of whole genome heterosis by localizing some of its building blocks into small overdominant genomic regions. Phenotypes that were correlated to the ultimate reproductive trait, seed number per plant, were considered components of reproductive fitness and showed a higher number and effect of overdominant QTL compared to nonreproductive phenotypes.

Many over-dominant effects were reproducible over several years and environments. For example, the mean fruit

Figure 2



Solanum pennellii IL heterosis. IL8-3 and IL2-5 were crossed to M82 (M) and the hybrids (H) and parents were evaluated for total fruit yield per plant (per M^2) in three growing seasons. In 2001 the plants were grown in a normally irrigated field (wet) and in dry conditions that received 10% of the water [28•]. The pyramiding of the two introgressions was achieved by crossing the ILs to generate a hybrid that is heterozygous for both genomic segments (Pyr).

yield of plants heterozygous for IL2-5 and IL8-3 was significantly higher than both parents in three years of testing in irrigated and drought conditions (Figure 2). The pyramiding of these heterotic introgressions further increased yield beyond the individual components, though in a less-than-additive manner [17,27]. The increased confidence in the stability of overdominant effects resulting from multiyear analyses has led to a major effort in detailed component phenotyping of smaller genomic intervals of the heterotic segment.

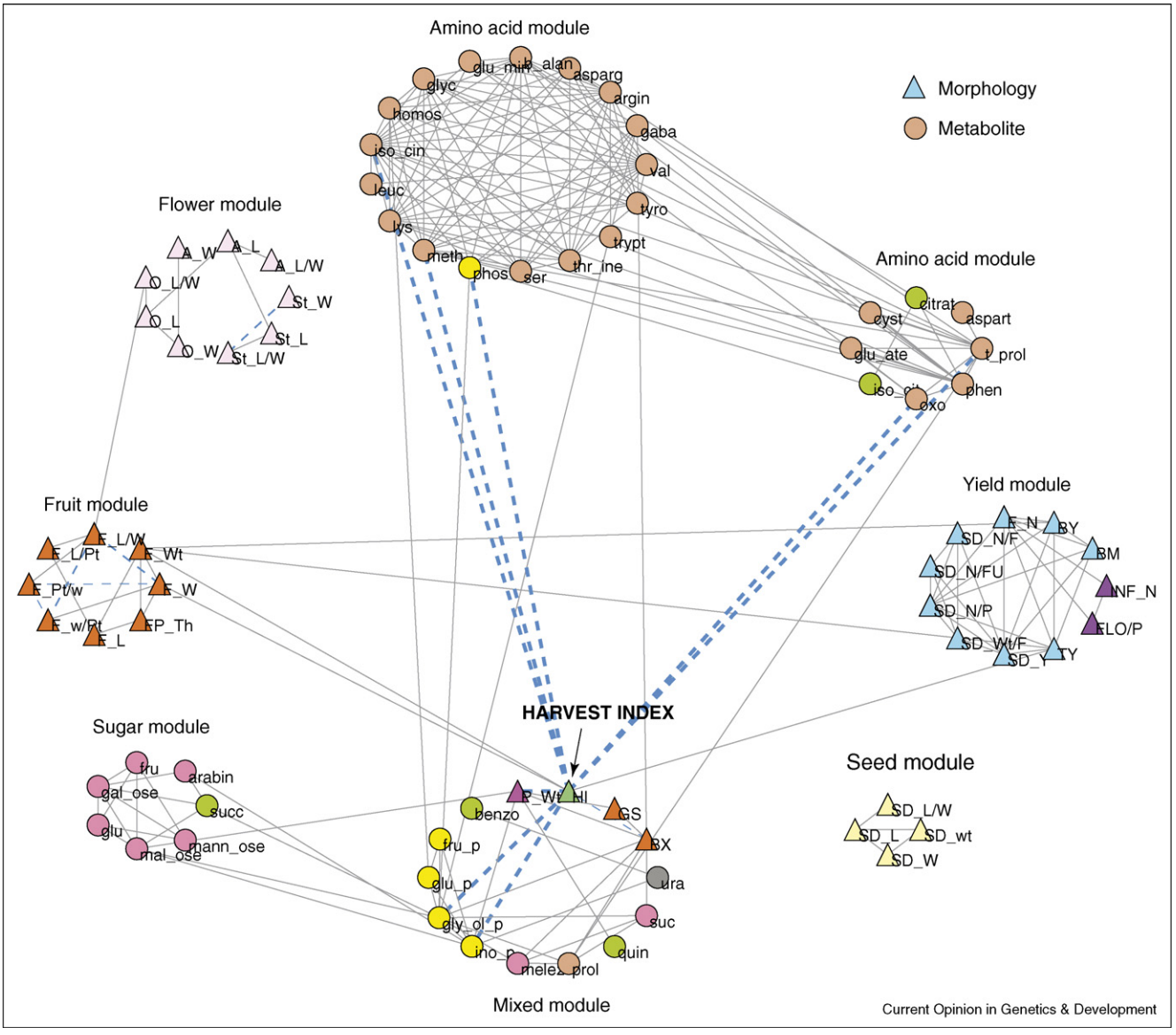
Importantly, IL heterosis has already progressed beyond scientific publications into practical use in agriculture. A QTL pyramiding study with three independent yield-promoting introgressions resulted in a hybrid with yields 50% higher than leading commercial varieties in multiple environments and irrigation regimes [28•]. Moreover, introgressions originating from *S. pennellii* were introduced into lines of processing tomato, and the resulting hybrid, AB2, is presently a leading variety in California (http://www.ptab.org/ranking9.htm), which is the largest world producer of industrial processing tomatoes. These real-world applications illustrate how 'exotic' alleles from interspecific diversity can enrich the genetic basis of cultivated plants to improve productivity.

IL resolution of integrated developmental networks

The *S. pennellii* ILs have recently gone beyond standard QTL identification studies, and ventured into a multifaceted systems-level analysis to address a classical biological question relating to plant architecture and physiology. A large-scale association study linking plant structure and biochemistry was carried out by phenotyping the ILs for a wide range of plant morphology and fruit metabolic profiles [29••]. An integrated cartographical network of these diverse phenotypes revealed morphology-dependent and morphology-independent links among a large number of fruit metabolism and yield associated QTL. One novel aspect of the analysis showed that Harvest Index (HI; Figure 3), which is a measure of the source–sink relationship between the vegetative and reproductive tissues, is the central hub in fruit metabolism. A similar negative correlation between biomass and certain seedling metabolites was shown recently in an Arabidopsis RI population, indicating a common resource availability bottleneck in plant development [30••].

The major pleiotropic IL affecting metabolic QTL was IL6-3. This IL carries a functional *SELF PRUNING* (*SP*) gene (orthologous to *Arabidopsis TERMINAL FLOWER1*)

Figure 3



A system view of IL-born morphology and metabolism interplay. Cartographic representation of the combined metabolic and morphological network of the tomato ILs [29]. Each trait (node) is represented by a shape (metabolites by circles and the phenotypes by triangles). The metabolites are color-coded according to type: brown, amino acids; pink, sugars; green, organic acids; and yellow, phosphates; grey, miscellaneous, and module names are defined according to the most prevalent trait type. A line connecting two traits represents a significant correlation between them. Correlation of all trait pairs was calculated using IL means (total of 76 lines); gray lines represent positive correlations, blue lines represent negative correlations (significance threshold of $P < 0.0001$). Harvest index (HI), the ratio of fruit yield to total plant mass (plant weight + fruit yield), is the central pleiotropic hub of the network.

that qualitatively reverts *sp* 'determinate' processing tomato varieties back to the 'indeterminate' vine-like sympodial growth that defines wild species. *SP* mutants exhibit compact growth by successively decreasing flowering time between sympodial units, making *SP* the most important developmental and agronomical switch to enable processing tomato production. As all *S. pennellii* ILs are in the background of the determinate line M82, the indeterminacy of IL6-3 immediately provided an isogenic perennial that facilitated the identification of *SP* by map-based cloning [31]. It is important to note that IL6-3 is the most QTL laden in the entire IL population, and it is believed that *SP*, having vast pleiotropic effects by modifying source–sink relationships, is the central link between plant morphology and fruit metabolite distribution. Interestingly, IL9-2-5 carries a second sugar QTL tightly linked to *Brix9-2-5*, which appears only in *SP* determinate mutant plants due to the semi-indeterminate

effects of another *SP* homologue, *SP9D* [32]. Together, these examples illustrate that with the continued development of Solanaceae genome resources, more detailed systems-level analyses will be possible, opening the door to new discoveries through extending the phenomic network.

Linking genomes, QTL and plant breeding

A bioinformatic challenge now facing quantitative genetics is to display detailed information about the components of the genetic and phenotypic variation in a form of statistical and graphical outputs in a unified ontology-based genomic framework [33•,34–36]. A first-generation *S. pennellii* IL-based working model that enables mapping QTL, viewing basic trends, associations between traits, and finding specific combinations of phenotypes is called 'Real Time QTL' [37] (http://zamir.sgn.cornell.edu/Qtl/Html/home.htm). Another database that exhibits *S. pennellii* IL data is the Tomato Metabolite Database (TOMET (http://ted.bti.cornell.edu/), which includes the expression profile data of more than 10 000 unique genes and composition data of approximately 60 metabolites that contribute to fruit flavor and human nutrition. The integration of such data, as is being achieved in mice [38], will allow for a wider view of plant biology as it relates to crop improvement.

Plant geneticists, realizing the value of wild species diversity to the genetic improvement of plants, have generated over the past decade numerous populations that segregate for genetically mapped complex phenotypic variation. IL populations are now available for many wild tomato species [39–43]. Similarly, the power of the IL approach is now being realized in other model systems, including major food and biofuel crops such as pepper [44], rice [45], barley [46], wheat [47], maize [48•], soybean [49] and Arabidopsis [50••]. These populations are being phenotyped widely, but only a small fraction of the raw data finds its way to existing databases. The same is true for an already large body of QTL mapping data on F2 and RIL populations where epistasis is measured more effectively. Achieving multilayered phenotypic integration in all model systems will be necessary in the future to realize the full discovery potential of genomics-assisted comparative QTL studies.

A proof of concept for an extended Solanaceae complex trait view has been the identification of an orthologous yield associated QTL common to potato and tomato: the invertase gene *invGE* colocalizes with cold-sweetening and starch content QTL from potato [51••]. The potato invertase is orthologous to the tomato *LIN5* that is responsible for the QTL *Brix9-2-5* [25], indicating that natural variation of sugar yield in tomato fruits and potato tubers is controlled by functional variants of the same gene. This example illustrates the potential of horizontally linking QTL biology with genomic resources across a wide phylogenic basis.

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- •• of outstanding interest

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