

## The genetics of fruit flavour preferences

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**Abstract** | Intensively bred fruit crops, including tomatoes and strawberries, are widely viewed as lacking flavour. The lack of breeder focus on the consumer is largely due to the genetic complexity of the flavour phenotype as well as a lack of a simple assay that can define consumer preferences. Rapid advances in genomics have opened up new opportunities to understand the chemistry and genetics of flavour. Here, we describe the underlying causes for the loss of flavour in fruits over time and delineate a blueprint for defining the chemistry of consumer liking, reducing that knowledge into a molecular roadmap for flavour improvement.

### Cultivar

A plant variety that has been produced in cultivation by selective breeding for desirable characteristics.

### Molecular breeding

A process broadly encompassing all aspects of molecular biology, including genetic engineering and genome editing, but more narrowly defined as the use of large-scale genomic data to define genetic differences between individuals in a breeding population. These polymorphisms are used to develop genetic markers that facilitate the rapid selection of cultivars with desired traits.

### Genome-wide association studies

(GWAS). Association mapping of a phenotype in a population with whole-genome DNA polymorphisms.

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For decades, people have complained about the flavour of commercial produce, and the tomato is a prime example of consumer dissatisfaction<sup>1–3</sup>. Flavour has not been a priority in breeding for most intensively bred commercial fruits. The customer of the breeder is the grower. When growers are paid solely on the basis of how much product they produce, the focus of a breeder must by necessity be on overall cost of labour, yield, resistance to pests and pathogens and postharvest handling. In most commercial production systems, growers are not paid for flavour quality, and they do not demand it. Moreover, the flavour phenotype is difficult to measure and is highly influenced by the environment<sup>4,5</sup>, and the underlying genetics are not well defined. In general, the end user, the consumer, has been left out of cultivar development. However, a focus on the consumer presents major opportunities to grow markets, particularly with a quality trait such as flavour. Consumers want agricultural products, especially fruits and vegetables, that taste good and are nutritious. The challenge ahead is to figure out how to provide higher quality products to consumers without compromising the agronomic traits that growers demand.

The best solution to this difficult paradigm lies in molecular breeding, whereby complex traits are reduced to sets of molecular markers. Steady improvements in technology and reductions in cost have made large-scale genome sequencing widely accessible, facilitating genome-wide association studies (GWAS). It is now possible to define the chemistry of consumer preferences (that is, liking) and identify genes regulating the synthesis of flavour chemicals, as well as the alleles of those genes that provide a more favourable chemical composition. Here, we discuss an integrated process starting with the consumer. This process integrates human sensory science, biochemistry, genetics and genomics to define the chemistry of fruit flavour and provides a blueprint for how to improve flavour quality. While we emphasize tomato,

where the most progress has been achieved to date, similar opportunities exist in other important fruit crops.

### What is flavour?

When we talk about the ‘flavour’ of a food, we naturally think of taste. But flavour is the sum of inputs from multiple senses that informs the brain what we are eating. Taste refers only to the five classes of receptors in the mouth that measure the levels of sweet, sour, salty, bitter and umami in a food. Smell (olfaction) is also essential to flavour perception<sup>6</sup> and is mediated by a large family of olfactory receptors in the nasal epithelium that recognize volatile organic compounds. More than 400 human olfactory receptor genes exist, and it is estimated that humans can distinguish, on average, 1 trillion different smells<sup>7</sup>. Hence, smell provides the diversity of flavours that we experience in our lives. Although texture and appearance also influence our perception of flavour, in this Review, we focus on flavour-associated chemicals that are present in fruits.

The range of chemicals contributing to the flavour of a fruit includes those that interact with taste receptors and those that are volatile and interact with the olfactory receptors. For taste, the most important are sugars, acids and, in some fruits, bitter chemicals. Nutrients, including sugars, typically act as cues for ripeness to attract seed-dispersing organisms<sup>8</sup>, and they accumulate to their highest levels in ripe fruits<sup>9</sup>. The most common fruit sugars are sucrose, glucose and fructose, although other sugars, such as sorbitol, are important in some species, such as apple and sweet cherry. The most common acids include citrate, malate and ascorbate. The volatile landscape is considerably more complex. Hundreds of volatiles can be detected in most fruits. For example, there are over 400 detectable volatiles in tomato<sup>10</sup>, 300 in apple<sup>11</sup> and 80 in kiwifruit<sup>12</sup>. Adding further complexity, many volatiles can also be converted to nonvolatile

**Volatile organic compounds**

Organic compounds that have a high vapour pressure at room temperature. High vapour pressure allows the chemical to partition into the gas phase. Volatile organic compounds are important contributors to flavour when they vaporize in the mouth and travel to the olfactory epithelium where they are recognized by specific sets of receptors.

**Principal component analysis**

A statistical method that is used to simplify a complex data set by transforming a series of correlated variables into a smaller number of uncorrelated variables called principal components.

**Retronasal olfaction**

The sensory modality responsible for flavour. Perception of volatiles generated within the mouth and transmitted to the olfactory epithelium.

sugar esters by uridine diphosphate (UDP)-sugar glycosyltransferases<sup>13</sup>, preventing them from contributing directly to flavour.

Progress in understanding the genetics of flavour has been slow. To identify the volatiles that have an impact on fruit flavour, many researchers focus on the concept of odour units and the log ratio of concentration of a volatile in the fruit to the human odour threshold<sup>14</sup>. In theory, if its concentration exceeds the detection threshold, the volatile must contribute to the flavour of a fruit. However, there are serious flaws with this theory. Human odour thresholds are very difficult to measure and vary widely between individuals<sup>15</sup> and the matrix in which the chemical is provided<sup>16</sup>. Reported odour thresholds for some chemicals vary by several orders of magnitude<sup>17</sup>. The theory also does not factor in mechanisms of odourant receptor chemistry. Receptors are modular; individual receptors recognize multiple volatiles, and a single volatile can be recognized by multiple receptor family members<sup>18</sup>. Thus, different volatiles act combinatorially to trigger responses at subthreshold levels. Also, there is likely to be an optimum level of each volatile, above which a volatile may have a negative impact on flavour. For example, tomatoes carrying the *malodorous* locus produce large amounts of phenylpropanoid volatiles<sup>19</sup>, but the taste of these tomatoes is uniformly disliked (D.M.T. and H.J.K., unpublished observation). Finally, although the odour unit calculation provides an approximation of whether a chemical contributes to flavour intensity, it reveals nothing about whether that chemical influences how much we like or dislike the food.

**Flavour preferences**

A large body of literature catalogues the contents of sugars, acids and flavour volatiles in a wide array of fruits (see REF.<sup>20</sup> for an excellent review of fruit flavour chemicals). The chemistry of each fruit is unique, imparting the specific flavours associated with the species and the cultivar. Yet, commonalities do exist. Sugars and acids are critical to the flavours of most fruits. Some volatiles, such as  $\gamma$ -decalactone and the six-carbon fatty acid-derived volatiles, are present in many different fruits, including peaches and strawberries<sup>21,22</sup>. Identifying the genes that encode biosynthetic enzymes should facilitate the identification of orthologous genes in other species. But maintenance and improvement of flavour in each species present unique challenges.

A major challenge is to identify not only which volatiles contribute to flavour but also, and more importantly, which contribute to liking. The most accurate way to determine which chemicals have an impact on liking is empirically, by providing consumers with a sample set as chemically diverse as possible, quantifying potential flavour chemicals with gas chromatography and high-performance liquid chromatography, including sugars, acids and volatiles, and statistically correlating each chemical with liking scores. Although large consumer panels and analytical chemistry are expensive, the results provide a qualitative and quantitative list of flavour chemicals associated with the preferences of panellists. For example, Tieman et al.<sup>23</sup> used a 100-person consumer panel to evaluate 150 different tomato

samples over 5 years, identifying a set of sugars, acids and 29 volatiles that are positively or negatively correlated with liking. Notably, the most abundant volatile in a tomato fruit, Z-3-hexenal, does not correlate with liking, emphasizing the weakness of reliance on odour units to prioritize targets for intervention. By contrast, volatiles such as 1-pentanol and E-2-pentenal, which are predicted to be below the threshold of detection<sup>14</sup>, make significant positive contributions to both overall flavour intensity and liking (TABLE 1). Principal component analysis shows close correlations of perceived sweetness and overall flavour intensity with liking. Flavour intensity is a measure of the contribution of volatiles through retronasal olfaction. An interesting conclusion emerging from the consumer studies is that some volatiles act by enhancing the perception of sweetness<sup>24</sup>; specific volatiles make the tomato taste sweeter than it actually is. This effect of volatile-enhanced sweet perception is observed in other fruits as well (described below).

On the surface, identification of 29 separate volatiles that affect consumer liking suggests that genetic improvement of flavour should be extremely challenging. But many of these volatiles are metabolically linked (FIG. 1). Interestingly, these volatiles are derived from a smaller set of primary metabolites that are all essential to the human diet<sup>25</sup>. For example, the essential amino acids phenylalanine, leucine and isoleucine are the precursors of 10 of the 29 volatiles, while the essential fatty acids linoleic and linolenic acid are the precursors of another 10.

A similar study in strawberries indicates that overall liking is associated with ratings of sweetness, flavour intensity and texture<sup>21</sup>, although the authors did not directly correlate specific chemicals with consumer liking (TABLE 1). Instead, they correlated 31 volatiles with flavour intensity and found that 38 volatiles significantly increased the perceived intensity of sweetness. A subsequent study directly correlated specific chemicals with consumer acceptance<sup>26</sup>. The authors reported significant positive correlations between 'acceptability' and perception of sweet aroma intensity and grouped sets of terpenoids and lactones. Unfortunately, because the two groups used different methods for collecting volatiles, there is not much overlap in the identified volatiles. However, where overlap exists, the results are consistent. For example, linalool positively correlated with flavour intensity, whereas  $\gamma$ -dodecalactone and Z-3-hexenyl acetate positively correlated with sweetness.

A 3-year study correlating the chemical contents of southern highbush blueberry with liking identified fructose, pH and several volatiles as being either positively or negatively correlated with consumer liking scores<sup>27</sup>. These authors also examined the genetic and environmental variability of each of these factors to identify a subset that represents the most logical targets for genetic intervention. As with tomato and strawberry, there is a strong correlation between liking, sweetness and flavour intensity (TABLE 1). While sweetness was a major factor determining liking, this score was not highly correlated to sugar content. None of the top five rated varieties were in the top five for sugar content. These results illustrate the important role that volatiles have in amplifying the perception of sweetness in blueberry.

Table 1 | Flavour chemicals and attributes significantly correlated with consumer preferences

Flavour components or attributes	Blueberry	Tomato	Strawberry
Flavour intensity	Positive	Positive	Positive
Sweetness	Positive	Positive	Positive
<b>Sugars</b>			
Fructose	Positive	Positive	Positive
Glucose	Positive	Positive	Positive
Sucrose	Positive	NR	Positive
<b>Volatiles</b>			
Hexanol	NSS	NSS	Positive
1-Methylbutyl butyrate	NR	NR	Positive
1,8-Cineole	Negative	NR	NR
2-Nonanone	Negative	NR	NSS
1-Nitro-2-phenylethane	NR	Positive	NR
1-Nitro-3-methylbutane	NR	Positive	NR
1-Penten-3-ol	NSS	NSS	Negative
1-Penten-3-one	NR	Positive	NR
2,5-Dimethyl-4-methoxy-3(2H)-furanone	NR	NR	Negative
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	NR	Positive	NR
2-Ethyl-hexan-1-ol	NR	NR	Positive
2-Heptanone	NSS	NR	Positive
2-Hexanone	NR	NR	Positive
2-Isobutylthiazole	NR	Positive	NR
2-Phenylethanol	NR	Positive	NR
3-Methyl-1-butanol	Positive	NSS	NR
3-Pentanone	NR	Positive	Positive
6-Methyl-5-hepten-2-ol	NR	Positive	NR
3-Ethyloctane	NR	NR	Positive
6-Methyl-2-heptanone	Negative	NR	NR
6-Methyl-5-hepten-2-one	NSS	Positive	Negative
Benzaldehyde	NR	Positive	NR
Benzyl cyanide	NR	Positive	NR
Butyl acetate	NR	Negative	Positive
Butyl butyrate	NR	NR	Positive
Z-2-pentenal	NR	NR	Positive
Z-2-penten-1-ol	Negative	NSS	NSS
Z-4-decenal	NR	Positive	NR
Z-Linalool oxide	NSS	NR	Positive
Decyl butyrate	NR	NR	Positive
Ethyl butyrate	NR	NR	Positive
Ethyl decanoate	NR	NR	Negative
Ethyl propionate	Positive	NR	NSS
Eugenol	NR	Negative	NR
$\gamma$ -Decalactone	NR	NR	Positive
$\gamma$ -Dodecalactone	NR	NR	Negative
Heptaldehyde	NSS	Positive	NSS
Hexyl acetate	NSS	Negative	Positive
Hexyl butyrate	NSS	NR	Positive

Table 1 (cont.) | Flavour chemicals and attributes significantly correlated with consumer preferences

Flavour components or attributes	Blueberry	Tomato	Strawberry
<b>Volatiles (cont.)</b>			
Isoamyl acetate	Positive	NSS	NSS
Isobutyl acetate	NR	Negative	NR
Isopentyl butyrate	NR	NR	Positive
Isopropyl butyrate	NR	NR	Positive
Methyl anthranilate	NR	NR	Negative
Isovaleraldehyde	Positive	NSS	NR
Isovaleric acid	NR	Positive	NR
Isovaleronitrile	NR	Positive	NR
Linalool	Negative	NR	NSS
Methyl butyrate	NR	NR	Positive
Nerolidol	NR	NR	Positive
Methyl salicylate	Negative	NSS	NR
Nonyl aldehyde	NSS	Positive	Positive
Nonyl 2-methylpropanoate	NR	NR	Positive
Pentyl butyrate	NR	NR	Negative
Phenylacetaldehyde	NSS	Positive	NR
Prenyl acetate	NSS	Negative	NSS
Salicylaldehyde	NR	Negative	NR
S-Methyl thiobutyrate	NR	NR	Positive
<i>E</i> -2-Heptenal	NR	Positive	NR
<i>E</i> -2-Decenal	NR	NR	Positive
<i>E</i> -2-Hexenal	Negative	NSS	Negative
<i>E</i> -2-Hexenyl butyrate	NR	NR	Positive
<i>E</i> -2-Octenal	NR	NR	Positive
<i>E</i> -2-Pentenal	Negative	Positive	Positive
<i>E</i> -3-Hexen-1-ol	NR	Positive	NR

Data taken from previously published consumer evaluation panels (REFS<sup>21,23,24,27</sup>). Positive or negative indicates a significant correlation with consumer liking scores. NR, not reported as being present; NSS, present but not significantly correlated with liking.

We can draw some conclusions from these studies. Perceived sweetness and flavour intensity are the major determinants of liking. Sugars are usually positively correlated with sweetness and liking, but not always. Volatiles have a considerable impact on sweetness, and fruits that are lower in volatile content are perceived to be less intensely flavoured, less sweet and less liked. Importantly, a notable positive effect on sweetness can be achieved independent of sugar content. Hence, it should be possible to greatly increase volatile content without negatively affecting yield, an approach that is not possible with sugar content, which correlates negatively with fruit weight<sup>28,29</sup>. Notably, different fruits contain different combinations of volatiles that modulate flavour (TABLE 1). That is, several chemicals, including sugars and volatiles, contribute to liking differently in different fruits. For example, 3-pentanone positively correlates with strawberry and tomato flavour<sup>21,23</sup>. By contrast, acetate esters, such as butyl acetate and hexyl acetate, positively contribute to liking of strawberry but are negatively correlated with

liking of tomato<sup>21,23</sup> (TABLE 1). Thus, while the building blocks of flavour are frequently shared across species, it is the sum of the parts that gives each fruit its unique flavour. Knowledge of pathways and genes encoding various enzymes will be useful, but how those pathways are regulated will frequently vary across different fruits, which needs to be taken into account as we try to manipulate flavour chemical composition.

### Breeding for flavour

Loss of flavour quality in the tomato fruit can be traced back to the earliest stage of human intervention and selection for ever larger fruits<sup>30</sup>. Linkage drag associated with selection for fruit weight genes has led to large changes in the fruit metabolome<sup>31</sup>. This trend has been further exacerbated in modern commercial field production by selection for the largest fruit in cultivars with heavy, synchronous fruit sets. Fruits with less sugar are simply not as flavourful.

The *uniform (U)* gene is illustrative of how we arrived at a less flavoursome tomato. The process of

### Linkage drag

A negative effect on some aspect of quality or plant performance upon backcrossing a gene into a different cultivar. Typically refers to negative effects associated with genes physically linked to the gene of interest. A particular problem when introgressing a trait (such as disease resistance) from a different sexually compatible species.

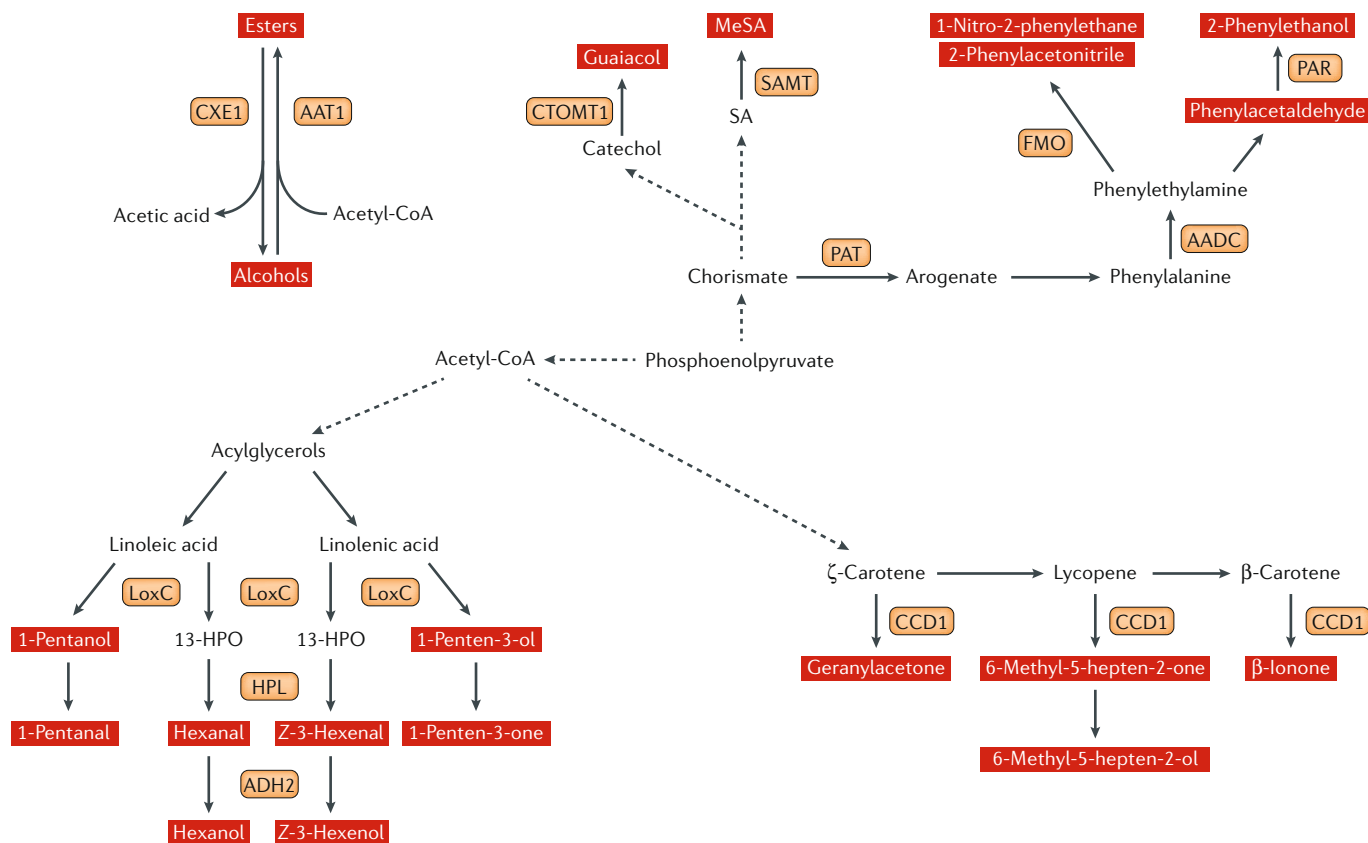


Fig. 1 | **Synthesis pathways for tomato flavour volatiles.** Solid lines indicate a validated step in a pathway with the responsible enzyme indicated in an orange box. Volatiles are indicated in red. Nonvolatile intermediates are indicated in black. Steps in which the responsible enzyme has not been defined are indicated with dashed lines. Broadly, volatiles are derived from the shikimate (for example, guaiacol), phenylpropanoid (for example, 2-phenylethanol), fatty acid (for example, Z-3-hexenol) or carotenoid cleavage (for example, geranylacetone) pathway. In addition, volatile alcohols can be reversibly converted to esters by the action of an alcohol acetyltransferase (AAT1) and a carboxymethyltransferase (carboxylesterase 1 (CXE1)). See REFS<sup>60–67</sup> for gene citations. 13-HPO, 13-hydroperoxide; AADC, aromatic amino acid decarboxylase; ADH2, alcoholdehydrogenase 2; CCD1, carotenoid cleavage deoxygenase 1; CTOMT1, catechol-O-methyltransferase 1; FMO, flavin-containing monooxygenase; HPL, fatty acid hydroperoxide lyase; LoxC, lipoxygenase; MeSA, methyl salicylate; PAR, phenylacetaldehyde reductase; PAT, prephenate aminotransferase; SA, salicylate; SAMT, salicylic acid methyltransferase.

ripening starts with internal tissue, subsequently progressing from the blossom end to the stem end. This phenomenon gives the appearance of an unripe dark green shoulder on an otherwise red fruit. In the early 20th century, the *uniform* mutation, which greatly reduces the contrasting green-shoulder phenotype, was identified. The industry recognized the value of a fruit that appeared to be uniformly more ripe, and today the mutant *uniform* allele is present in virtually all modern commercial cultivars, although the responsible gene was identified only recently. *U* encodes a golden 2-like transcription factor whose loss of function results in fruits with fewer chloroplasts<sup>32</sup>. Because chloroplasts are the factories that convert carbon dioxide to sugars, and a considerable portion of fruit sugar is synthesized in fruit chloroplasts, the net effect is to lower fruit sugar content in *u/u* mutant fruit. Thus, a mutation introduced into commerce to make fruit more visually appealing effectively reduced flavour.

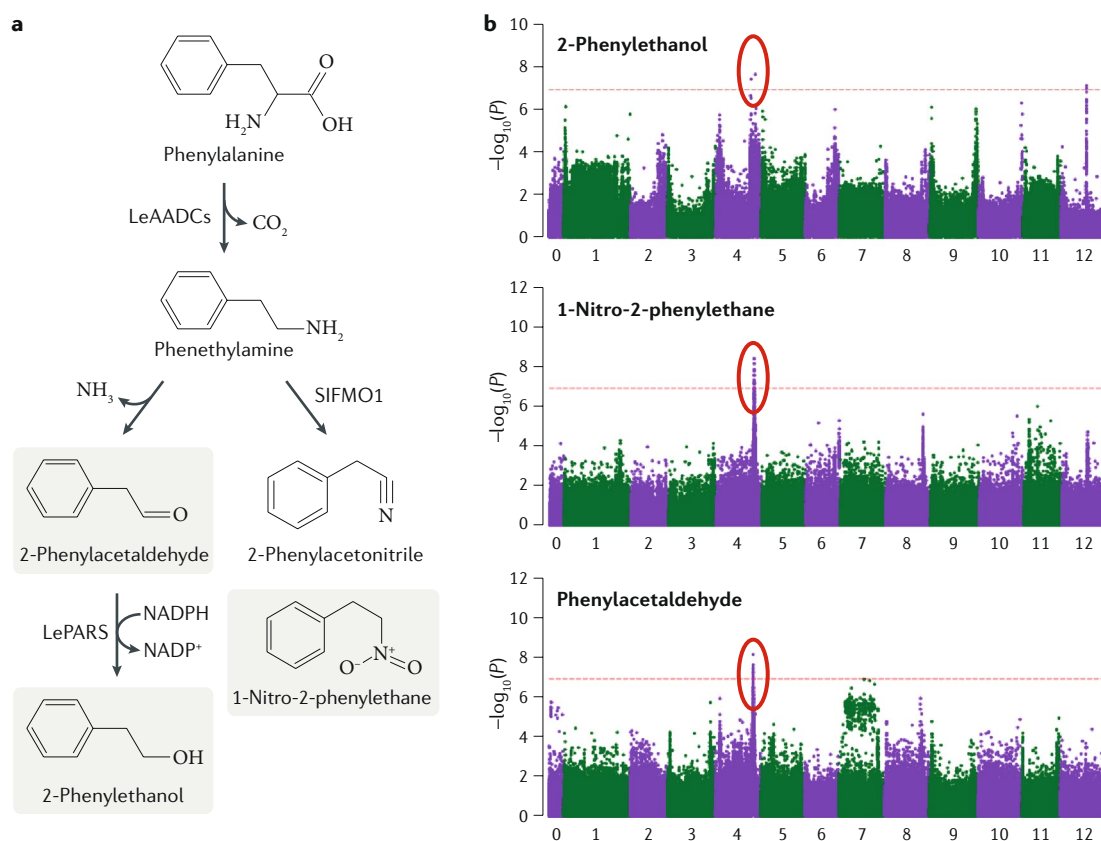
Breeders have not deliberately ignored flavour in their selections. Flavour is a difficult phenotype to score. Fruit flavour chemical composition can be highly variable. It is influenced by genetics, environment and agronomic practices, making it difficult to objectively evaluate even a single cultivar over different seasons or locations. Because individual tastes vary, flavour should be evaluated by as many different people as possible. These consumer panels are expensive and not amenable to high throughput; only a limited number of samples can be evaluated in a single panel. Scaling methods introduce variability between individuals and across sessions. There are also human genetic and ethnic factors that influence preferences<sup>33</sup>. Nevertheless, there are scaling techniques available that compensate for individual differences<sup>34</sup>. Using appropriate methodology, one can correct for person-to-person and session-to-session variability in a way that makes it possible to statistically model the flavour chemical composition of an average ‘ideal’ fruit averaged over the sampled population. It is important to recognize, however, that this ideal fruit may not be the most liked by every individual.

**Introgression lines**

(ILs). A genetic line that contains a gene or region of a chromosome from one species in the genome of another. It is created by repeated backcrossing of an interspecific hybrid with one of its parents.

Consumer panels are evidently not a practical solution for a typical breeding programme in which many different plants are being evaluated in a season. So how do we reduce flavour to a practical assay? After identification of the appropriate set of chemicals and the target concentrations, assays can quantify those chemicals. However, while sugars and acids are relatively straightforward to measure, volatile quantification is technically demanding and expensive. Quantification relies upon a dedicated gas chromatograph and availability of pure standards for each chemical. Most fruits have many volatiles; in tomato, several hundred volatiles can be detected<sup>10</sup>, and accurate quantification of the most important volatiles cannot be automated. Very few breeders have access to these resources. The ideal assay would reduce flavour to a defined set of molecular markers that can be incorporated into the standard battery of markers used for inheritance of disease resistance, fruit colour and shape, and growth habit. To achieve this goal, it is necessary to identify the genes (or at least genetic loci) that modulate the synthesis of the important flavour chemicals, as well as the most desirable alleles of those genes in donor material.

**Progress in tomato flavour improvement.** Over the past decade, progress towards understanding the regulation of flavour chemical synthesis has largely been at the level of single genes and pathways (FIG. 1). Some genes have been identified based on insights into the biochemistry of metabolic pathways. Introgression lines (ILs) derived from crosses between domesticated tomatoes (*Solanum lycopersicum*) and related wild species, including *Solanum pennellii*<sup>35</sup> and *Solanum habrochaites*<sup>36</sup> have been very useful, as have segregating populations derived from crosses between different *S. lycopersicum* cultivars<sup>37–39</sup>. This investment has led to a reasonable understanding of many of the metabolic pathways and identification of important biosynthetic enzymes<sup>40–44</sup>. The gene-by-gene approach to elaborating volatile synthesis has been facilitated by the development of a high quality reference genome for *S. lycopersicum*<sup>45</sup> and the sequence of *S. pennellii*<sup>46</sup>, as well as precise mapping of the *S. pennellii* IL population by transcriptome profiling<sup>47</sup>. Nonetheless, this piecemeal approach is time consuming, requiring isolation and mapping of recombinants and validation of function in transgenic plants.



**Fig. 2 | Identification of a locus controlling synthesis of phenylalanine-derived volatiles. a** | In tomato, 2-phenylethanol, 1-nitro-2-phenethane and phenylacetaldehyde (highlighted in grey boxes) contribute to flavour intensity and consumer liking. Phenylalanine is converted to phenethylamine by a family of aromatic amino acid decarboxylases (AADC). The pathway then splits. Removal of ammonia by an as yet uncharacterized amine oxidase leads to formation of phenylacetaldehyde, which in turn is converted by phenylacetaldehyde reductase (PAR) to 2-phenylethanol. In parallel, a flavin monooxygenase (SIFMO1) is essential for synthesis of 2-phenylacetonitrile and 1-nitro-2-phenylethane. This pathway is not fully elaborated. **b** | Manhattan plots derived from genome-wide association studies indicate a locus that coordinately influences the abundance of the three volatiles shaded in grey in part a. Red circles indicate the position of this locus on chromosome 4 that rises above the significance threshold (indicated by the dashed red line). Part b is adapted with permission from REF.<sup>23</sup>, AAAS.



### Heirloom varieties

Older varieties that have been maintained for some desirable attribute (for example, flavour, colour and shape). Although there is no legal definition of 'heirloom', it is generally considered to be inbred. Heirlooms usually lack the performance and disease resistance found in modern cultivars.

### Quantitative trait locus (QTL)

A region of a chromosome that quantitatively influences a measured phenotype. That region, defined by polymorphic molecular markers, contains one or more physically linked genes causative of the phenotype.

### Selection bottleneck

A substantial enrichment for a specific subset of genes with reduced allelic variation relative to the variation found in a species. Loss of diversity through intensive breeding with a small population.

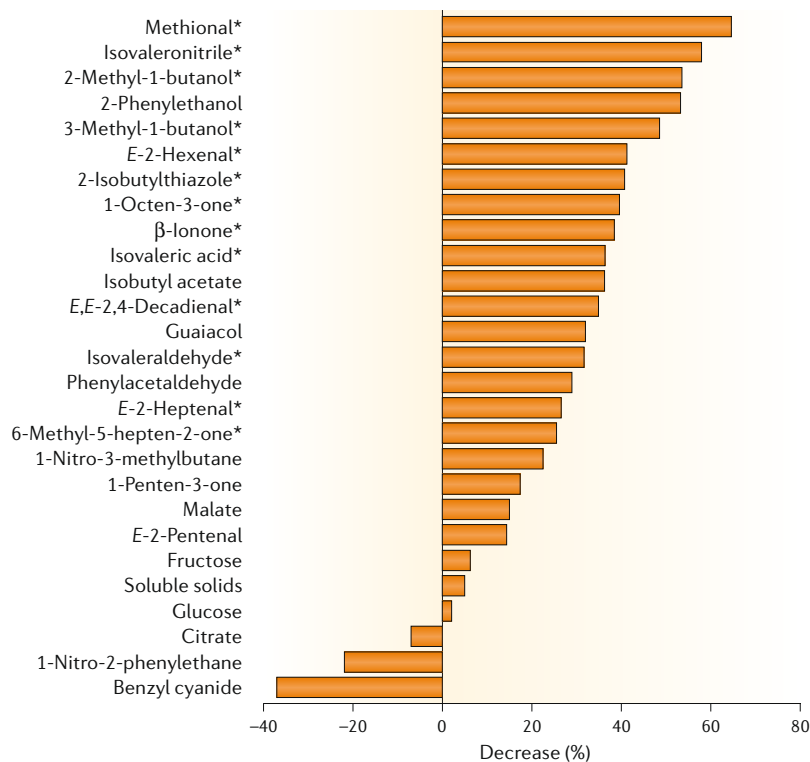
Greatly reduced costs for DNA sequencing have transformed the flavour landscape. Access to almost unlimited numbers of genomes has made GWAS possible. Assuming sufficient phenotypic diversity in the population, loci affecting flavour chemicals can be identified. Tieman et al.<sup>23</sup> used a population of 400 cultivars ranging from wild *S. lycopersicum* var. *cerasiforme* and heirloom varieties to modern commercial hybrids to identify genetic loci that affect many of the most important sugars, acids and volatiles. Consistent with knowledge of the biosynthetic pathways, many of these loci coordinately control multiple metabolically related chemicals (FIG. 2). Reducing a quantitative trait locus (QTL) to the causative gene is still labour-intensive, but to date, multiple single nucleotide polymorphisms (SNPs) have been identified that define loci substantially affecting fruit flavour chemical composition. Some of the QTLs define important metabolic functions. For example, a SNP associated with sugar content changes a single amino acid in an extracellular invertase, creating a kinetically superior enzyme<sup>21</sup>. The gene encoding this invertase, *LIN5*, was previously identified as the causative gene underlying a sugar QTL in an *S. pennellii* IL population<sup>28</sup>. An aluminium-activated malate transporter associated with fruit acid content was also identified. A similar approach was performed by Bauchet et al. in 300 tomato varieties<sup>48</sup>. Using SNP markers, the authors identified 79 loci influencing multiple

primary and secondary metabolites, several of which overlap with those identified by Tieman et al.<sup>23</sup>.

**Evolution of tomato fruit quality.** The breeding history of each species uniquely affects its flavour genetics. Tomato and strawberry, for example, have been intensively bred, and the commercial germplasm is not representative of the genetic diversity available within the species<sup>23,49</sup>. Modern cultivars have gone through an extreme selection bottleneck, and most contain a small subset of the allelic variation present in the species at most of the flavour chemical loci defined by GWAS<sup>30</sup>. On average, they have significantly lower contents of approximately 50% of the important flavour volatiles (FIG. 3), which is precisely what we would predict if there was random selection of desirable and undesirable alleles in the absence of positive selection<sup>23</sup>. GWAS have identified exactly which inferior alleles for synthesis of each volatile are present in the modern population. Superior alleles can be substituted using molecular breeding. Because volatiles are biologically active at nanomolar to picomolar concentrations, it should be possible to reintroduce the favourable alleles without considerably compromising agronomic performance. Because minimal-to-no yield loss is essential for commercial adoption, we are at present limited to volatile improvements. Although we know precisely how to increase sugar content in tomato, introducing superior alleles would almost certainly reduce yield and/or fruit size. Nonetheless, because olfaction is the major part of consumer liking, we can expect that restoring volatiles to levels present in heirloom varieties will result in substantial flavour improvement.

**Progress in other fruit crops.** Next to tomato, probably the most characterized fruit crop with respect to flavour is strawberry<sup>50,51</sup>. In addition to sugars, multiple volatiles have been associated with consumer liking<sup>21</sup>, and several biosynthetic genes have been identified. Indeed, one of the first genes associated with fruit volatile synthesis was identified in strawberry<sup>52</sup>. One example of flavour loss relates to the volatile methyl anthranilate (MA). MA is a major flavour note in wild diploid strawberries that has been lost in almost all octoploid commercial cultivars. Volatile quantification in fruits from an F1 population resulting from a cross between an MA producer and a non-producer combined with a bulked-segregant transcriptome analysis led to identification of a methyltransferase responsible for MA synthesis<sup>49</sup>. Identification of the gene, *ANTHRANILIC ACID METHYL TRANSFERASE (FanAAMT)*, is an important step towards restoration of MA in commercial cultivars. A similar example concerns the strawberry volatile  $\gamma$ -decalactone, which is also correlated with flavour preferences<sup>53</sup>. Differential gene expression was used to screen a population segregating for the presence or absence of  $\gamma$ -decalactone in fruit. A gene encoding a putative biosynthetic enzyme, *FaFAD1*, was found to be absent from all cultivars that do not produce  $\gamma$ -decalactone. A molecular marker that tracks the gene was developed and can now be incorporated into flavour breeding programmes.

Flavour genetics has been extensively examined in apples, and the availability of large numbers of genomes



**Fig. 3 | Average changes in flavour-associated chemicals in modern tomato varieties.** Modern varieties were identified using discriminant analysis of whole-genome sequences as described in REF.<sup>23</sup>. Compositional differences are expressed as a per cent decrease of modern varieties relative to heirloom *Solanum lycopersicum* varieties. \*Indicates chemicals that are significantly different between the two populations. Figure is adapted with permission from REF.<sup>23</sup>, AAAS.

## Backcrossing

Recurrent crossing to a parental variety in order to introduce specific genetic loci into an otherwise isogenic line.

has enabled the mapping of multiple flavour-associated loci. In one study, whole-genome sequences of 117 *Malus* accessions from 24 related species were used to document the effects of domestication on the apple genome<sup>54</sup>. Among other traits, the authors looked at loci affecting flavour, principally sugar and acid accumulation. Within selected regions were multiple candidate genes affecting sugar transport and synthesis, as well as a previously identified locus encoding a malic acid transporter at the *Ma* locus<sup>55</sup> that is orthologous to the malate transporter identified by GWAS in tomato<sup>23</sup>. A genome-wide SNP association study identified multiple loci associated with different classes of volatiles, including esters<sup>56</sup>. Potential causative genes included an alcohol acetyltransferase gene, *MdAAT*, that synthesizes volatile esters. Yet another GWAS study identified QTLs affecting broad classes of volatiles, including esters, alcohols, aldehydes and phenylpropanoids<sup>57</sup>. These results are consistent with a prior SNP association study including loci containing the gene *AAT1*, which encodes alcohol acyl transferase, and *ACSI*, the gene encoding the ethylene biosynthesis enzyme 1-aminocyclopropane-1-carboxylate synthase (*ACC*). Together, these findings indicate that orthologous genes from different species function to synthesize the same flavour volatiles. Thus, functional identification of genes in one species will surely facilitate applications in other species.

One of the most extensive association studies to date was performed in cucurbits. Cucurbitacins are a class of cyclic triterpenes that affect bitterness in cucumbers. Zhou et al.<sup>58</sup> used comparative analyses of the genomes of cucumber, melon and watermelon

to identify genes responsible for synthesis of distinct cucurbitacins, as well as transcription factors regulating their synthesis. It is an outstanding example of exploiting comparative genomics to elucidate a metabolic pathway; in this case, one related to important flavour and pharmaceutical chemicals.

## The road ahead

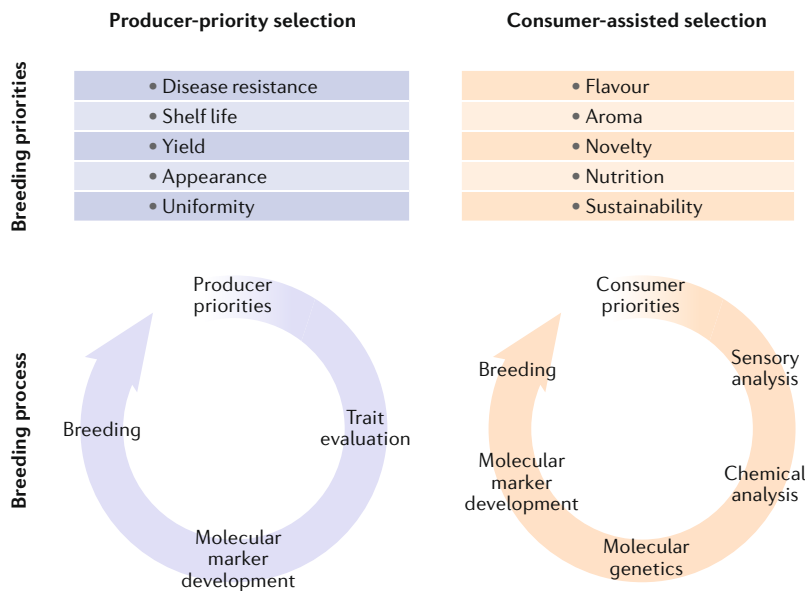
The focus on producer rather than consumer traits is often raised as one of the largest mistakes made by the biotechnology industry with genetically engineered plants over the past two decades. Consumers are dissatisfied with the flavour of many fruits, and substantially improved flavour quality is an opportunity to grow markets and improve consumers' diets.

Flavour quality is a complex trait that has deteriorated in intensively bred crops because the tools for maintenance and improvement have not been available. The fix requires a long-term investment. We call that investment 'consumer-assisted selection' (FIG. 4). The key is to start with the consumer, using appropriate tools to assess what they like<sup>59</sup>. After defining the target (for example, great flavour), we define the underlying biochemistry and genetics, identifying allelic variation to recreate that target and provide the breeder with the resources to build the product. That product is then validated by the consumer. For tomato and strawberry, the targets have been largely defined for the US market. But assembling products using molecular breeding tools without sacrificing yield is still a major task.

Flavour is a polygenic trait, and we do not know how many favourable alleles one must reintroduce to perceptibly increase flavour in a modern commercial cultivar. This question can be answered only empirically. However, knowing that it is possible to increase multiple volatiles with single genes, it is reasonable to assume that considerable flavour improvements can be achieved with only a few genes. Each market segment will have a somewhat different target composition, in turn creating product opportunities.

We have the knowledge and the tools to achieve major flavour improvement in tomato and many other fruit crops through genetic engineering. Indeed, genetic engineering could cut years off breeding programmes in slow-maturing tree fruits, such as apple or peach. While improved flavour would likely be well received by a majority of consumers, the barriers to commercial introduction may be too large to be practical. The large financial cost of regulatory approval, especially for export crops, with the uncertainty of how such a product would be received by the public is very risky. Another challenge with a crop such as tomato is that it is produced seasonally across a wide geographic area, owing to different environments and disease pressures, necessitating the introduction of transgenes into many different cultivars. In most nations, each transgenic event is considered a separate product, with its own regulatory cost. Backcrossing, a single deregulated event into multiple elite inbred lines, would delay widespread introduction into the market by years.

Today, allele replacement using genome editing is not easily accomplished in tomato or other fruit crops,



**Fig. 4 | A roadmap for consumer-assisted genetic selection.** Most breeding programmes focus on producer-driven traits (left panel). The consumer-assisted process (right panel) starts with the consumer, determining what they desire in the product, working through the chemistry of consumer preferences, the underlying genetics, through to molecular-assisted breeding. Finally, the product of the breeding programme is cycled back to the consumer before release. Figure is adapted from REF.<sup>68</sup>, CC-BY-4.0 (<https://creativecommons.org/licenses/by/4.0/>).



although this will hopefully change in the near future. Generating gene knockouts, however, is fairly straightforward. Some volatiles are negatively correlated with liking in tomato, including methyl salicylate, guaiacol and several acetate esters<sup>15</sup>, and knocking out genes in their biosynthetic pathways could improve flavour. This strategy makes sense since transformation and regeneration of elite inbred lines is possible in some of these crops, including tomato and strawberry. Genome editing has the potential to immediately and cleanly introduce a trait with zero linkage drag and no backcrossing, and as such, genome editing will be an important complement to marker-assisted breeding programmes. As the technology advances to the point where allele swapping becomes routine, it is reasonable to expect that this form of ‘precision breeding’ will

largely replace backcrossing, at least in countries open to the technology.

### Conclusions

Tremendous progress has been achieved in defining the chemistry of consumer preferences of fruit crops. The explosion of inexpensive, high-quality genome information has provided a foundation for elucidating the genetics underlying this highly complex trait. A process leading from defining consumer preferences through delivering varieties with appropriate genetic makeup has been delineated. Reducing flavour to a molecular toolbox can and will be accomplished in the near future, promising products that should increase consumer satisfaction and demand.

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