



Revealing plant cryptotypes: defining meaningful phenotypes among infinite traits

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The plant phenotype is infinite. Plants vary morphologically and molecularly over developmental time, in response to the environment, and genetically. Exhaustive phenotyping remains not only out of reach, but is also the limiting factor to interpreting the wealth of genetic information currently available. Although phenotyping methods are always improving, an impasse remains: even if we could measure the entirety of phenotype, how would we interpret it? We propose the concept of cryptotype to describe latent, multivariate phenotypes that maximize the separation of *a priori* classes. Whether the infinite points comprising a leaf outline or shape descriptors defining root architecture, statistical methods to discern the quantitative essence of an organism will be required as we approach measuring the totality of phenotype.

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Introduction

What is phenotype? It is used to mean types of organisms, or to refer to specific traits [1]. It is malleable enough to encompass complex morphological organs, behaviors, and even types of molecules and their expression levels. Another attribute of phenotype is that all of the aforementioned attributes are potentially plastic, and can change under differing environmental conditions. They change temporally as well, including during the development of an organism. Phenotype can even be ecological in nature, describing biotic interactions between an organism and its environment.

Efforts attempting to systematically measure multiple scalar traits have been dubbed *phenomics* [2,3]. Like *genomics*, *phenomics* adequately captures the scale of

comprehensively phenotyping organisms, having been defined as the ‘systematic study of phenotypes on a genome-wide scale’ [2] and the ‘acquisition of high-dimensional phenotypic data on an organism-wide scale’ [3]. Although adequate for conveying the high-dimensionality of the organismal phenotype, the implied analogy with *genomics* is misleading in other aspects. For example, many genes may make up a genome, but no similar relationship exists for phenome. In this sense, there is no equivalence between genotype and phenotype. A gene is a physically defined entity, and even in a hereditary sense, it is an indivisible quantum. Together, many genes constitute a genome. Contrastingly, a trait is merely a facet, one of many arbitrary ways of measuring phenotypic reality. Traits are a subset of phenotype, but many traits do not reconstitute a phenome the same way genes additively produce a genome.

We suggest the term *holophenotype* to mean the totality of an organism’s phenotype (i.e., its physical, biological reality). Although aspects of a holophenotype can be measured in a given context, it can never be completely measured because it is infinite; inexhaustible environmental conditions and the limitless temporal space within an organism’s lifetime assure this. Yet it is important to define this reality we know exists but can never measure. *Holophenotype* and *phenome* may be viewed complementary: *holophenotype* is the ultimate phenotypic reality we attempt to measure, while the *phenome* is the measured product of phenomic, phenetic, or phenometric efforts measuring many traits.

The most meaningful phenotype would be multivariate in nature, and measurable if we had a holophenotype (or something approximate). What is most meaningful? We propose the term *cryptotype* to describe latent, multivariate phenotypes that maximize the separation of *a priori* classes. Whereas *pheno-* means ‘to show,’ *crypto-* is the opposite, ‘hidden’ or ‘secret.’ *Phenotype* and *cryptotype* are already used in linguistics to refer to overt and covert categories, respectively [4], the latter describing the combinative power of words that occur more frequently together than by chance [5].

We use cryptotype here similarly: cryptotype is the combination of individual traits that maximizes the separation of types. The principle is not unlike Fisher’s discriminant analysis [6] and elaborated upon by others later with respect to multidimensional biological problems [7]. Cryptotypes describe those features that maximize the

Glossary

Trait: A phenotypic attribute of an organism, usually (but not always) singular or univariate in nature.

Phenotype: Literally 'to show' the type. The collective traits of an organism, although may also refer to a specific trait or groups of traits.

Holophenotype: Literally 'whole' phenotype. Refers to the entire physical-temporal being of an organism. As infinite traits comprise holophenotype, it will never be measured fully.

Cryptotype: Literally the 'hidden' type. A latent combination of traits that maximize separation of *a priori* groups, whether genetically, developmentally, or in response to the environment.

Principal Component Analysis (PCA): Used most often as a dimension reduction technique to efficiently explain phenotypic variance as orthogonal, uncorrelated principal components.

Discriminant Function Analysis (DFA): Predicts a dependent categorical variable using one or more independent continuous variables. Useful in discriminating and classifying known groups with associated multivariate data.

Multivariate Analysis of Variance (MANOVA): Predicts multiple continuous dependent variables using one or more independent categorical variables.

Time-Frequency Analysis: Methods used to identify signals from frequencies that may change over time by analyzing both frequency and duration simultaneously.

Elliptical Fourier Descriptors: A method to explain closed contours that first compresses shape information to a chain code representation followed by extracting Fourier coefficients.

Generalized Procrustes Analysis (GPA): A method to compare multidimensional point data through translation, rotation, reflecting, and scaling, allowing for the superimposition and comparison of morphometric landmark data.

quantification of differences by genotype, environment, or developmental context, and therefore best reveal the underlying effects of these parameters on an organism. Cryptotype is inherently subjective, reflecting the question at hand and the scale of measurement. A cryptotype is always present for a given inquiry, but is hidden until the measurement of phenotypic space is exhausted and trait combinations that maximize the power to discriminate classes defined.

Digital and time-resolved phenotyping, as well as sequencing, proteomic, and metabolomic technologies, have vastly increased the comprehensiveness of data collection in many biological systems. These represent important advances, but are not the focus of this review. Rather, we ask: if the holophenotype of an organism were quantified, how would we interpret it in a biologically meaningful manner? The current impetus of high-throughput phenotyping is to measure myriad traits. But after the compendium of countless phenotypes is amassed, what then?

The cryptotype concept is especially relevant to plants. Above ground, there exists an abundant diversity of leaf morphs, each an outline comprised of infinite points. Below ground, intricate growth defines dynamic root topologies. The sessile lifestyle of plants means they respond to their environment through growth over time, and iteratively produce organs, demanding a continuous, ever-changing interpretation of phenotype. Below, we

describe the application of the cryptotype concept to leaves, roots, and time. We end with perspectives on the integration of phenotype across organs and levels of organization, the relevance of cryptotype to evolutionary questions, and the ambiguity of interpreting phenotype at individual and group levels.

Leaves: a spectrum of shapes

Shape is a microcosm of holophenotype. Within the curves of an outline is an infinite set of points. What methods can comprehensively describe the multivariate nature of shape, and to what degree do genetic, developmental, and evolutionary cryptotypes modulate shape in independent ways, additively contributing to the outline of a single leaf (Figure 1)?

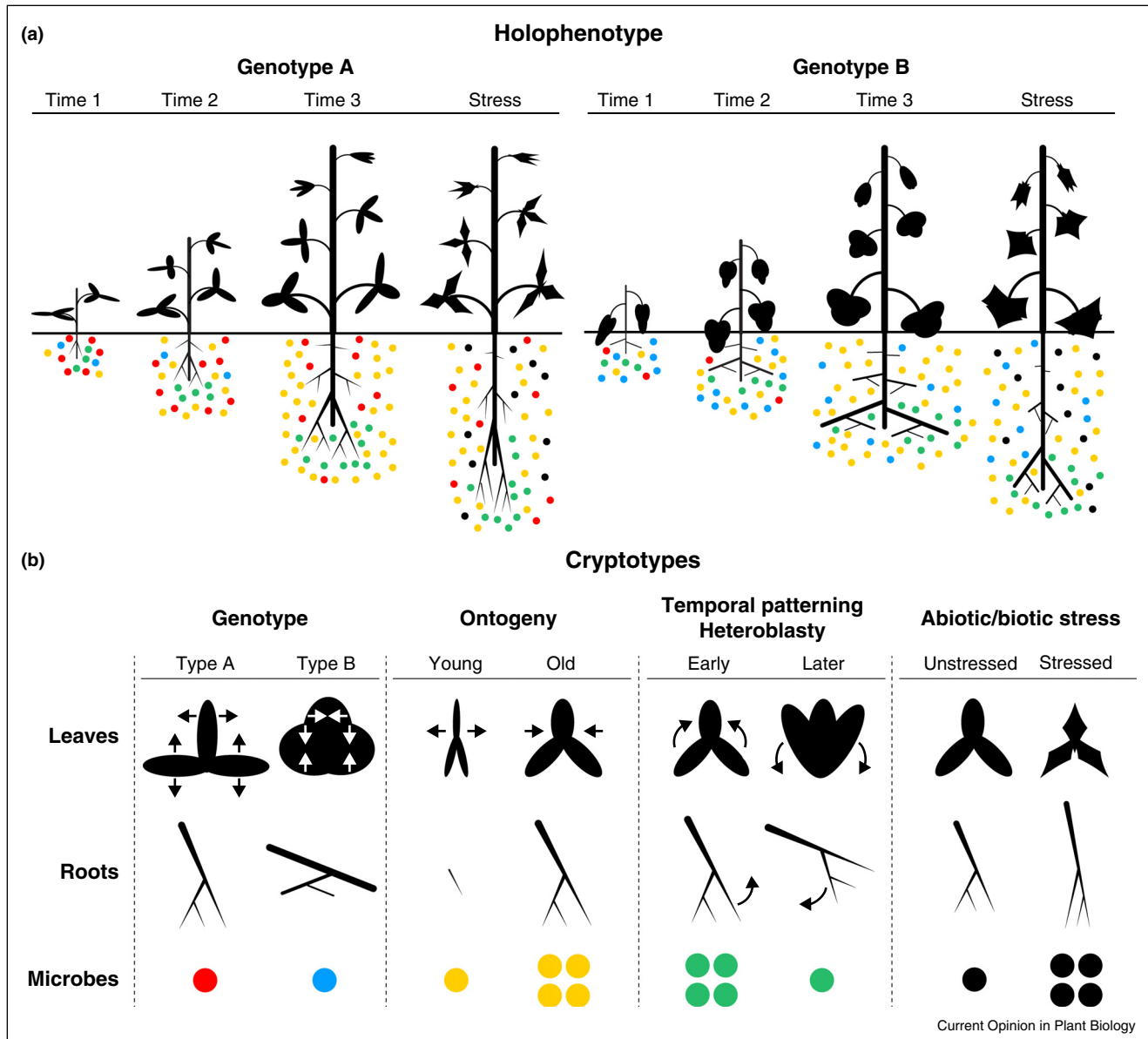
A biologically powerful approach is to leverage homology. For example, every grape (*Vitis vinifera*) leaf has five lobes and (*x,y*) coordinates can be assigned to lobe tips and sinuses [8]. A Generalized Procrustes Analysis (GPA) [9] is used to superimpose multiple coordinates before performing a dimension reduction technique, such as Principal Component Analysis (PCA), to define those combinations of coordinates that most efficiently explain the shape variance. Shape attributes most defining different levels of factors can be discerned using discriminant analysis [6], and sometimes different factors, such as genetic versus developmental, modulate shape independently [10].

Alternatively, a few landmarks placed on homologous coordinates (such as the base and tip of a leaf) can anchor equidistantly placed pseudo-landmarks to more fully describe shape. Pseudo-landmarks in snapdragon (*Antirrhinum majus*) describe cryptotypes that define shape attributes most varying by allometry (shape variability correlated with size) [11], mutation [12], heteroblasty (the changes in leaves arising from successive nodes) [13], and evolution [14**].

A more continuous description of shape can be achieved using Elliptical Fourier Descriptors (EFDs), especially when no homologous points between samples exist [15]. Converting shape into a numeric vector called chain code (a lossless data compression method), a Fourier analysis transforms shape into a set of harmonic coefficients [16*,17*]. EFD analysis of tomato leaves reveals ontogenetic [18], heteroblastic [10], asymmetric [19], and genetic [20] leaf cryptotypes, explaining distinct shape attributes that are modulated by different, orthogonal principal components.

Leaf shape is an excellent example how disparate biological factors — developmental, genetic, evolutionary, and environmental — jointly contribute to the holophenotype. Within the outline of a single leaf are numerous cryptotypes, requiring decomposition to observe and

Figure 1



An example of cryptotypes defining genetic, developmental, and environmental phenomena. **(a)** Plants exhibit different leaf shapes, root architectures, and microbiomes varying by genotype, plant or organ age (ontogeny), temporal patterning (e.g., heteroblastic changes in the shape of leaves arising from successive nodes), or environmental stress. Each of the above factors modulates phenotype in a multivariate fashion independent from each other. Comprehensively measuring phenotype and finding those traits that most discriminate factor levels can discern cryptotypes associated with each factor. **(b)** Cryptotypes defining multivariate attributes of plant phenotypes. In this example, the width of leaflets is modulated by genotype, overall leaf width varies by organ age (ontogeny), the shapes of leaves arising from successive nodes (heteroblasty) differ by the angles between leaflets, and unstressed versus stressed leaves vary by the concavity of leaflet shape. Note that the shape of each leaf is a summation of its genetic-spatio-temporal-environmental context and the effects of each cryptotype on shape. Similarly, cryptotypes contributing to root architecture and microbiome composition are shown.

measure their distinct effects on leaf morphology (Figure 1).

Roots: dynamic topologies

Root systems offer a different set of morphometric challenges than leaves. As complex three-dimensional

(3D) branching networks, both topological and geometrical features can be considered, including relative orientation within the soil, and developmental progression, which is heavily influenced by environment [21–23]. Our relational understanding of how these features contribute to root system architecture and function is naïve at best.

A major challenge to uncovering root cryptotypes is accurately capturing root architecture traits. Focusing on young, relatively simple root systems has allowed entire three-dimensional morphologies to be digitally reconstructed for several species and their natural variants [24,25,26,27]. These digital models allow shifts in the phenotyping paradigm from single measurements to multiple shape descriptors that may comprehensively capture architecture [28,29]. Whereas single descriptors can explain only a small fraction of phenotypic variability, their combined effects on discerning genetic effects on root morphology may be identified using machine learning [30] and modified MANOVA [27,31] (Figure 2). Once multivariate QTL are identified, Discriminant Function Analysis (DFA) parses allelic effects at each locus into weighted combinations of descriptors that reveal their otherwise hidden contributions to overall root shape; that is, genetic cryptotypes [27] (Figure 3). Similarly, PCA and biplot clustering of multivariate data sets has been used to statistically define functional root shape classifiers [32].

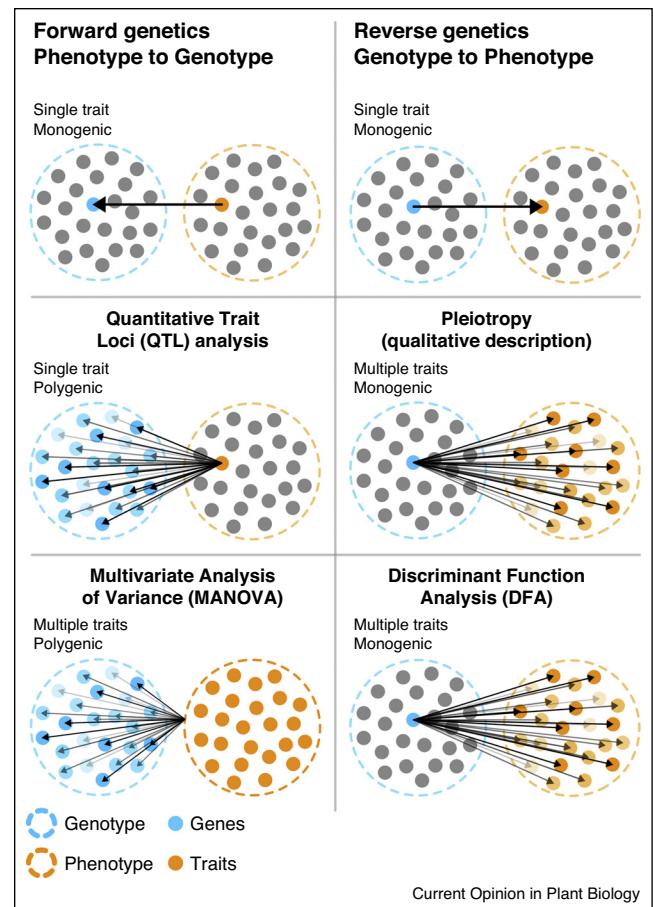
Numerous efforts have been made to mathematically deconvolve complex root shapes into ‘rooted tree’ graph structures, and identify the elementary rules governing their patterning [23,33–35], but this information remains elusive. Mature roots systems, which may span many feet or more, and which may contain hundreds of thousands of root branches [36], each in a micro-environmental space, are perhaps irreconcilably complex to understand from such top-down approaches. However, as multivariate field [37] and laboratory data sets continue to parameterize increasingly sophisticated structure-function models [38,39] or emerging deformable domain models [40], bottom-up approaches may in fact allow the discovery of hidden variables that condition root growth and morphological complexity (Figure 3).

Time: infinite-dimensional characters

Time is implicit to the concept of cryptotype. Every plant phenotype exists within space-time, although phenotypes are not often examined along the time continuum. In some cases the relevant frequencies are minutes or less; in others they are entrained by circadian factors, or by seasonal cycles. The terms ‘infinite-dimensional characters’ [41], or later ‘function-valued traits’ [42], were proposed to portray the actual infinite space from which phenotype emerges, where phenotypic values are measured as a function of age. The visualization of the genetic basis of traits measured continuously over time is stunning [43], and reveals the shortcomings of the all too prevalent conception of phenotype as static. No model of phenotype is complete without considering time.

Time-frequency analysis can be used to understand phenotypes that do not integrate smoothly over time, that is, they are rapid and dynamic and do not have a regular periodicity — for example a root responding to a local

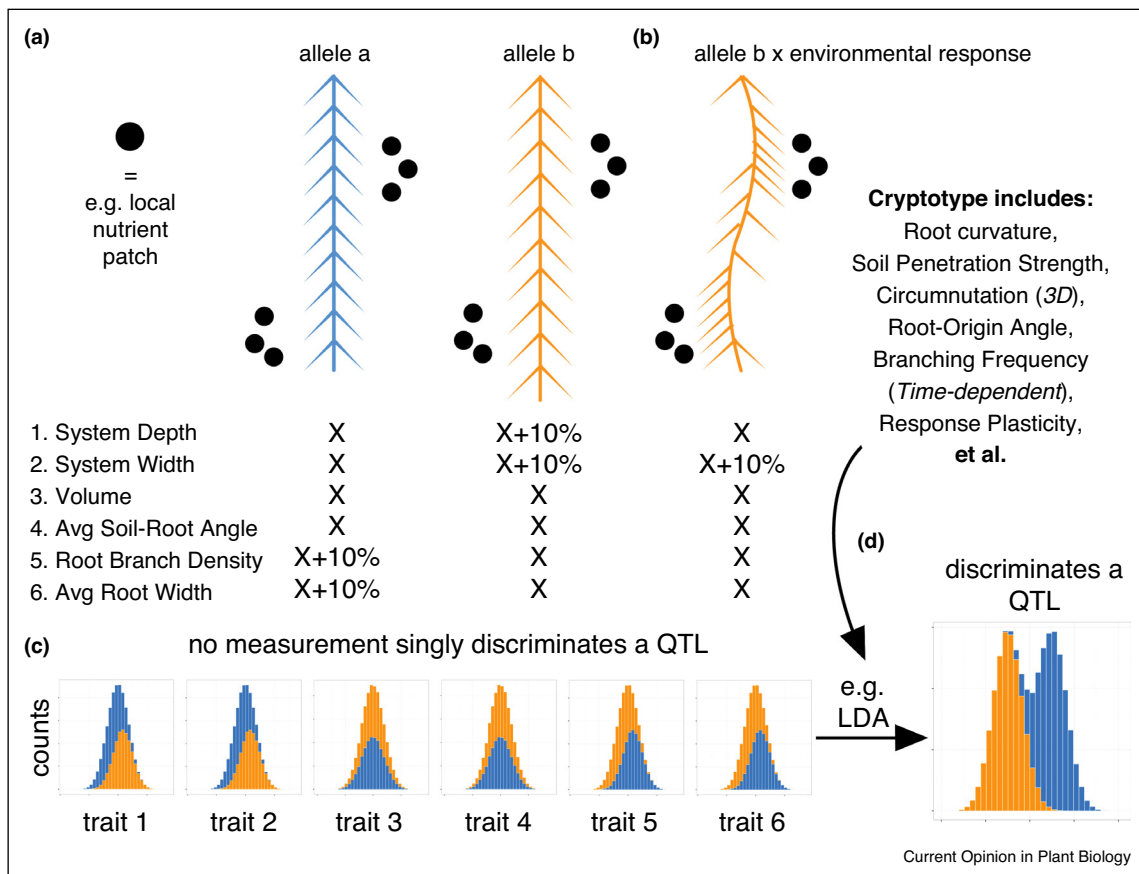
Figure 2



Univariate and multivariate relationships between genotype and phenotype and statistical methods. Shown are relationships between genotype (blue circle outline) and phenotype (orange circle outline), comprised of genes (solid blue circles) and traits (solid orange circles), respectively. Arrows indicate directional relationships between genes and traits. The simplest relationship between genotype and phenotype is the monogenic contribution to a single trait, whether studied from a forward or reverse genetic perspective. Traditional Quantitative Trait Loci (QTL) analysis determines the polygenic basis underlying a single trait. The opposite of QTL analysis is pleiotropy, in which the multivariate phenotypic effects of a single gene are discerned, but often in a qualitative fashion. QTL can be determined for multivariate traits using methods such as Multivariate Analysis of Variance (MANOVA). Likewise, Discriminant Function Analysis (DFA) can analyze traits most discerning different gene variants (i.e., the genetic cryptotype), making qualitative assessments of pleiotropy rigorous and quantitative.

stimulus. After collecting many dense (relative to scale) time series data sets, dimension reduction and clustering techniques can be used in conjunction with wavelet or Fourier analysis to identify dynamic processes influencing a phenotype. In this way the effects of maternal factors, developmental progression, and growth plasticity can be deconvolved [44]. Or, similarly, subtle effects of genetic mutation can be resolved along the time axis [45,46].

Figure 3



Combining subtly discriminating phenotypes increases discrimination of QTL effects. **(a)** The superficially similar phenotypic effects of allele a (blue) or b (orange) in a given environment can be measured as small differences in individual traits. **(b)** Assumes a plasticity response to local nutrient patches in allele b versus no response for allele a. The groups of individual traits become less discriminating in this case because of the increased influence of hidden traits on root morphology. **(c)** An example of where individual traits poorly discriminate allele effects at a locus that influences root growth, but when combined into an linear discriminant analysis (LDA), more strongly identify an underlying QTL. **(d)** Identifying and measuring the effects of phenotypes relevant to the cryptotype would greatly increase the potential for QTL identification.

New approaches for genome-wide genetic analysis of function-valued traits have incredible potential to identify the genetic basis of multi-scalar *processes* by treating time continuously rather than discretely [47]. Numerous different methods, for example continuous shape transforms or growth-response curves, can be used to generate function-values, which are then resolved into QTL by statistical models such as logistic-mixture [47], or average/maximum LOD over time [43*,48**]. Compared with traditional QTL mapping that proceeds stepwise through time intervals, function-value mapping has greater power to reveal the true multi-genic nature of phenotype [48**]. This knowledge will have profound implications for our fundamental understanding of how hidden variables drive phenotype. For example, the concept of ideotype [49], or model-based plant characters ideally suited to an environment, could be recast in the light of plasticity and process-based traits.

Conclusions

The concept of cryptotype, leveraging combinations of traits that maximize the discrimination of leaf outlines, root architecture, or dynamic changes in phenotype over time, is already implemented to discern genetic, developmental, and environmental contributions to multivariate phenotypes. What of the integration of such complex traits, across organs and with underlying molecular and cellular biology? Non-cell autonomous effects contribute to whole plant phenotypes and often the causal basis of traits lies in unexpected places. In tomato, leaf morphology and roots modulate fruit metabolites [20,50]. Although we have focused on morphological phenotypes in this commentary, ultimately such traits are derived from complex gene expression, proteomic, and metabolomic signatures [51], and integration of morphology and physiology with molecular and cellular attributes is required to quantify the holophenotype. Experimental

designs to insure adequate power measuring so many phenotypes over time and their interactions will be key, and tools to facilitate such large-scale experiments may ultimately come from agricultural industries [52].

The cryptotype concept is more than just a comprehensive method for quantifying phenotype, and has implications for evolutionary theory as well. The genetic basis of domestication is described piecemeal: beyond glumes, branching, juvenility, among others [53] the entirety of maize was transformed from its ancestors. How would our understanding of the genetic basis of maize domestication differ if the holophenotype were measured? Would it be the same as adding up the genetic basis of individual traits, or would there be significant epistasis and additional polygenicity? Would the effect size of existing QTL decrease? When applied to natural selection, cryptotype may yield similar insights. If some traits are neutral but other selected, how does our perspective change when traits are treated combinatorially, and how does a comprehensive view of phenotype alter the concept of constraint [54]?

The limits of a multivariate approach to phenotype, and the defining traits that comprise cryptotypes, are seemingly boundless. If studying epigenetic phenomena, does phenotype become transgenerational? If all the individuals that compose a population are measured, do those traits that are constant and unvarying constitute ‘wild-type’? How does the concept of phenotype apply to groups, their collective behavior and interactions [55]? How do phenotypes propagate across levels of organization as emergent properties? Currently, comprehensive genotypic characterization is commonplace and readily achievable. Yet the measurement of the totality of phenotype — the holophenotype — for a single organism has never been achieved and is the ultimate limiting factor to biological understanding.

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