Extending the Concept of Diversity Partitioning to Characterize Phenotypic Complexity

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Abstract: Most components of an organism’s phenotype can be viewed as the expression of multiple traits. Many of these traits operate as complexes, where multiple subsidiary parts function and evolve together. As trait complexity increases, so does the challenge of describing complexity in intuitive, biologically meaningful ways. Traditional multivariate analyses ignore the phenomenon of individual complexity and provide relatively abstract representations of variation among individuals. We suggest adopting well-known diversity indices from community ecology to describe phenotypic complexity as the diversity of distinct subsidiary components of a trait. Using a hierarchical framework, we illustrate how total trait diversity can be partitioned into within-individual complexity (α diversity) and between-individual components (β diversity). This approach complements traditional multivariate analyses. The key innovations are (i) addition of individual complexity within the same framework as between-individual variation and (ii) a group-wise partitioning approach that complements traditional level-wise partitioning of diversity. The complexity-as-diversity approach has potential application in many fields, including physiological ecology, ecological and community genomics, and transcriptomics. We demonstrate the utility of this complexity-as-diversity approach with examples from chemical and microbial ecology. The examples illustrate biologically significant differences in complexity and diversity that standard analyses would not reveal.

Keywords: multivariate, differentiation, chemical defense, functional diversity, plasticity, variation.

Introduction

Most phenotypic components are comprised of multiple traits collectively operating as an integrated unit (Pigliucci and Preston 2004; Swallow and Garland 2005). Phenotypic complexity is an intuitive concept, but it can be difficult to define (e.g., Horgan 1995). “Complex” is generally defined as consisting of interconnected or subordinate parts (Swallow and Garland 2005); more complex phenotypes have more interconnected parts than less complex phenotypes. Examples of complex phenotypes include behavioral syndromes (Sih et al. 2004), biomechanical systems (Wainwright 2007), chemical defenses (Jones and Firn 1991), and metabolic pathways (Hamberger and Bak 2013). Complex phenotypes might characterize individual cells, organ systems, organisms, social groups, or ecosystems.

Here we apply an existing approach to a new conceptual arena by using the diversity partitioning analyses of community ecology as a general framework for quantifying phenotypic complexity. We illustrate how trait diversity can be partitioned into distinct within-group versus among-group components of variation at multiple hierarchical levels all the way down to the level of individual complexity. For a given hierarchical level or grouping, each diversity estimate (within group, among group, or a pooled total) describes a unique aspect of the phenotype in an intuitive and biologically meaningful way. We demonstrate the appeal of the complexity-as-diversity approach with examples of multivariate chemical defenses and soil microbial traits that are important in nutrient and energy flow.

For some aspects of an organism, it is natural to express complexity as the diversity of subordinate parts. In the interest of clarity, we use phenotype to refer to a multivariate set of traits and, generally, not as the total set of all traits comprised by an organism (e.g., Lynch and Walsh 1998). The framework we present here is particularly suited for analyzing complex phenotypes whose constituent traits can be intuitively conceived as counts or amounts. Obvious examples include cell or tissue types, gene expression patterns, ecological metabolomics, chemical defenses, and the proportion of time devoted to various behaviors. We view it as complementary to more traditional multivariate approaches, including those derived from quantitative genetics theory.

Traditional Analytical Approaches and Their Shortcomings

As phenotypic complexity increases, so does the challenge of analyzing and summarizing that complexity in intuitive yet biologically relevant ways. For example, many chemi-
Partitioning of Phenotypic Complexity

A New Conceptual Approach: Complexity as Diversity

Two distinct traditions have approached complexity in a similar way. Studies of the evolution of organismal complexity have used the number of part types to represent complexity at a given level of organization. For example, the number of genes in a genome (Kuo et al. 2009), the number of kinds of intracellular structures (McShea 2000), and the number of cell types (McShea 1996; Bell and Mooers 1997) have been used as general measures of organismal complexity. Likewise, ecologists describe ecosystems with few interacting species as simple and those with many interacting species as complex (May 1973; Ings et al. 2009). Both traditions point to a strong conceptual connection between diversity and complexity formalized in information theory (Rényi 1961; Bonchev 2003), and ecologists have used this formalism to develop a quantitative approach to species diversity (Hill 1973).

The principle behind this connection is that we can measure the complexity of a system or structure by the amount of information needed to describe it (Shannon 1948; Kolmogorov 1965), just as the diversity of a set can be measured by the amount of information needed to describe its composition. We can characterize a low-diversity system by measuring or identifying just a few of its elements; looking at a new element provides little new information. For a high-diversity system, each new element might provide a lot of new unexpected information about the whole. For example, we do not learn much by finding a pine in a pine forest; finding a rare orchid might add a lot to our description of the community, but it happens rarely. This principle underlies Shannon’s $H$ (Shannon 1948), which is the average information gained by randomly sampling and identifying one item. If all types are rare, each new sample provides a lot of unique information; the average ($H$) is large. If the system is mostly one type, each new sample is likely to be more of the same; the average information is small.

The same principle applies to organismal phenotypes. Consider the thought experiment of randomly sampling cells, messenger RNA, or pheromone molecules from an individual. If repeated sampling from the same individual gives the same tissue type, transcript, or chemical compound, we conclude that the organism’s phenotype is relatively simple. If new samples are often previously unobserved tissue types, transcripts, or compounds, we conclude that the individual’s phenotype is relatively complex. For

The strategies mentioned above are overtly designed to reduce the dimensionality (complexity) of the data. Instead of analyzing phenotypic complexity, the first step is to avoid it or place it into a black box. Although simplification is almost always a necessary aspect of analysis, simplification can sometimes be achieved in ways that illuminate, rather than obscure, interesting components of complexity. Diversity partitioning is one way to explicitly account for both the qualitative and quantitative aspects of phenotypic complexity, and it does so while providing easily interpretable and biologically meaningful estimates that describe complexity.
example, Jason et al. (2005) used diversity indices to summarize the diversity and equitability of terpenes in pine trees. However, they did not comment on the generality of the approach or the potential for hierarchical partitioning.

Here we apply the familiar conceptual framework of community diversity to describe multivariate phenotypes as the diversity, or effective number, of distinct subsidiary traits making up an individual’s phenotype. We then illustrate how that diversity can be partitioned into distinct within-group versus among-group components of variation at multiple hierarchical levels. For a given hierarchical level or grouping, each diversity estimate (within-group, among-group, or pooled total) describes a unique aspect of phenotypic complexity in an intuitive and biologically meaningful way. We demonstrate the appeal of the complexity-as-diversity approach with examples of multivariate chemical defenses and phenotypes of soil microbes associated with plant rhizospheres.

We emphasize that the complexity-as-diversity approach should be viewed as complementary to traditional multivariate methods, because each is likely to expose different aspects and patterns of a particular data set. As described here, the approach is entirely descriptive. We are not inventing any new calculations; rather, we are promoting the use of established frameworks to analyze novel kinds of data.

**Interpreting Effective Numbers**

The main idea of this article (complexity as diversity) is not tied to a particular computational program or mathematical representation of diversity. To make the discussion more concrete and provide methodological background for the examples, we outline one specific approach that is based on methods that have recently become popular in community ecology (Hill 1973; Jost 2007) as well as in physics and economics (Rényi 1961; Patil and Taillie 1982; Tsallis 2001).

Ecologists typically quantify species diversity using indices such as richness (the number of species), average information (Shannon entropy), or the probability of identity (Simpson concentration). With simple transformations (Rényi 1961; Hill 1973), these indices all express the diversity of a community \( D \) as numbers equivalents (Patil and Taillie 1982) or the “effective number” of species (Mac-Arthur 1965). Numbers equivalents describe the number of equally likely or common components (e.g., expressed genes, chemical compounds, and species) needed to obtain a given value of that index. Numbers equivalents have intuitive behaviors and interpretations, because they are in biologically relevant units of complexity with doubling properties (Hill 1973). For example, say a chemical ecologist has two equally diverse yet chemically distinct samples (i.e., samples with no shared compounds), each with effective compound number \( X \). If those two samples were combined, the chemical diversity of the combined sample should become \( 2X \) (Jost 2007). When diversities are expressed as numbers equivalents, their magnitudes have simple interpretations as the number of equally common phenotypic components. Likewise, a numbers equivalent describing the \( \beta \) diversity between these two samples should be two, because they are two completely distinct sets of chemicals. Thus, a numbers equivalent–based approach immediately provides a biologically interpretable measure of complexity and variation, unlike most multivariate or distance-based approaches.

Many commonly used diversity indices have numbers equivalents; where they differ is in their sensitivity to unequal amounts of the different subunits, indicated by the diversity order \( q \) (Hill 1973; Jost 2006). The phenotypic diversity \( (D) \) of order \( q \) when there are \( K \) traits (analogous to the \( S \) species of communities) is defined for \( q \neq 1 \) as

\[
D = \left( \sum_{i=1}^{K} p_i \right)^{1/(1-q)},
\]

where \( p_i \) is the relative amount of the \( i \)th trait (Jost 2006). The phenotypic diversity is undefined for \( q = 1 \), but its limit exists and equals the exponential of Shannon’s information index:

\[
D = \lim_{q \to 1} D = \exp \left( -\sum_{i=1}^{K} p_i \log p_i \right).
\]

Diversity of order 0 (richness) is completely insensitive to quantitative variation; only the presence or absence of a phenotypic component is considered. When \( q = 1 \) (exponential of Shannon’s \( H \)), each component is weighted by its frequency or relative abundance. For values of \( q > 1 \) (e.g., inverse Simpson concentration when \( q = 2 \)), more abundant phenotypic components become increasingly influential. Because all of the different orders are in units of effective numbers, we can use the differential sensitivity to unequal abundances to graphically assess phenotypic unevenness as a function of order \( q \) (Hill 1973; Jost et al. 2010).

In diversity profile plots, phenotypes with completely equal abundances of each subsidiary component will have the same effective number for any order \( q \); such a profile appears as a horizontal line in the plot (i.e., example I in fig. 1). Sharply descending curves (example III in fig. 1) indicate high unevenness (e.g., chemical phenotypes consisting of a few compounds of high concentration and many compounds of low abundance, or tissues with a few highly expressed genes and several genes with minimal expression). Effective numbers, when combined with diversity
heterogeneously distributed within and across organisms, level complexity, because complexity of part types is often perfectly capture notions of organism-level or ecosystem-

Diversity or number of part types at a single level does not
tend to underestimate the true population \( \gamma \) and \( \beta \). Moreover, comparisons among study systems would be con-

lineages, or landscapes. Approaches that quantify the number of components or the hierarchical organization of parts are more satisfying. Community ecologists have a long tradition of partitioning a region’s total species diversity into two components: a measure of the average within-location or within-community diversity (\( \alpha \)) and a measure of the among-location or among-community differences in species composition (\( \beta \); Whittaker 1972; Lande 1996; Jost 2007).

Just as communities are partitioned into within-group versus among-group components, we can do the same for complex phenotypes. The \( \alpha \) diversity (\( ^a\alpha \)) is the within-

individual component. When using effective numbers, \( \alpha \) diversity can be interpreted as the effective number of individual phenotypic elements in a sample (e.g., the effective number of genes transcribed within a tissue or the effective number of deterrent compounds that make up a plant’s antiherbivore defenses). The \( \gamma \) diversity (\( ^\gamma \gamma \)) is the total effective number of constituent parts of a pooled group (e.g., an experimental plot, population, or species pool).

The among-group diversity component, \( \beta \) diversity, has two different interpretations depending on whether partitioning is additive (Lande 1996; Veech et al. 2002) or multiplicative (Jost 2007; Chao et al. 2012). In an additive partitioning framework, additive \( \beta \), or diversity excess, is calculated as \( ^\beta \beta = ^\gamma \gamma - ^a\alpha \) and quantifies the effective number of group-level phenotypic components not found within a typical sample (Chao et al. 2012). Additive \( \beta \) is in units of the effective number of parts, as are \( \alpha \) and \( \gamma \), and thus it reflects the magnitude of the difference between groups or samples. For example, if a population of 10 plants is chemically defended against herbivores with a \( \gamma \) diversity of 10 effective defensive compounds and an \( \alpha \) diversity of seven effective defensive compounds per plant on average, under the additive partitioning framework, \( ^\beta \beta = 10 - 7 = 3 \) effective defensive compounds. That is, effectively, three defensive compounds are in the population in excess of those present in a typical plant.

In the context of multiplicative partitioning, \( \beta \) diversity (\( ^\beta \beta = ^\gamma \gamma / ^a\alpha \)) is interpreted as the effective number of completely distinct phenotypic combinations present within a hierarchical level and estimates the extent of differentiation among phenotypes (e.g., the effective number of completely distinct chemotypes, or unique chemical cocktails, that an organism might use to defend against natural enemies within a population; Jost 2006, 2007). Using the hypothetical plant defense example above, multiplicative \( \beta \) would be \( \beta = 10/7 \approx 1.43 \) effective chemotypes (i.e., there would be 1.43 unique combinations of defensive chemicals in that population).

As defined above, \( \gamma \) and \( \beta \) are dependent on the number of individuals (\( N \)). Estimates based on a sample will tend to underestimate the true population \( \gamma \) and \( \beta \). Moreover, comparisons among study systems would be con-

Figure 1: Diversity profile plot illustrating the relationship between effective phenotype diversity (\( D \)) and diversity order (\( q \)). In this example, six traits have been evaluated. There are three samples (I, II, and III), each differing only in the evenness of subsidiary trait quantity. When \( q = 0 \), traits are weighted equally regardless of differences in trait abundance, and each phenotype has six effective traits at this order. As the diversity order increases, traits with higher abundances become more influential, and less weight is given to traits of low quantity. In example I, the relative amounts of each component are equal, and the diversity profile is a horizontal line (\( D = 6 \) for all \( q \)). Example III is dominated by two traits comprising approximately 60% and 20%, respectively, of the total abundance, and so the profile curve descends sharply before leveling out at approximately two effective traits. Example II is intermediate between I and III; the curve indicates mild disparity among components.

profile plots, facilitate intuitive comparisons of differences in the magnitude of qualitative variation and the distribution of quantitative unevenness for complex phenotypes.

Characterizing complexity as diversity provides familiar and intuitive measures based on information theory. When used properly, these can be compared fairly across systems (Jost 2006, 2007). In addition, this approach leads naturally to hierarchical analysis; the complexity of individual phenotypes can be understood in the same framework as the diversity among individuals in a population, differentiation between populations, and so forth.

Phenotypic Complexity and Hierarchical Diversity Partitioning

Diversity or number of part types at a single level does not perfectly capture notions of organism-level or ecosystem-level complexity, because complexity of part types is often heterogeneously distributed within and across organisms,
founded by differences in \( N \). To provide a standardized estimator of turnover (\( \epsilon_T \); Jost 2007), \( \beta \) can be transformed to

\[
\epsilon_T = \frac{\epsilon \beta - 1}{N - 1} = \frac{\epsilon \beta^*}{\alpha(N - 1)}.
\]

(3)

This differentiation measure expresses \( \beta \) diversity as a fraction of its maximum possible value given \( N_i \); it is zero when samples are identical and is 1 when every phenotypic combination is distinct. In the hypothetical plant example, the turnover is approximately 0.048. Note that this is a global measure that can be sensitive to sampling. See Chao et al. (2012, 2014) for an in-depth treatment of measures of similarity and differentiation.

**Level-Wise versus Group-Wise Partitioning**

Diversity partitioning has been extended to more than two levels (Crist et al. 2003). The general strategy for hierarchical partitioning is illustrated in figure 2. The key feature of adopting this approach for phenotypes is that the complexity (\( \alpha \) diversity) of the individual organism or structure is being expressed in the same framework as the variation among individuals and among groups of individuals (e.g., populations, regions, or experimental groups). This emphasizes interpretation of the variation in a data set in terms of partitioning or turnover (Anderson et al. 2011; Chao et al. 2012) of numbers of phenotypic elements, rather than more abstract multivariate distances.

We describe the approach proposed by Crist et al. (2003) as level-wise partitioning. Suppose that we have \( i = 1, 2, 3, \ldots, m \) sampling units (e.g., individuals, demes, regions, \ldots, continents). The total diversity is partitioned at the highest level as \( \gamma = \beta^m + \alpha_m \). The average diversity within subunits is further partitioned as \( \gamma = \beta^m + \beta^{m-1} + \alpha_{m-1} \), and so forth:

\[
\gamma = \alpha_i + \sum_{i=1}^{m} \beta_i^*.
\]

(4)

Similarly for multiplicative partitioning,

\[
\gamma = \alpha_i \prod_{i=1}^{m} \beta_i.
\]

(5)

This level-wise partitioning approach gives one \( \beta \), one \( \beta^* \), and one \( \alpha \) for each level (Crist et al. 2003). Level-wise \( \alpha \) is calculated from the average diversity within samples at hierarchical level \( i \),

\[
\alpha_i = D(\bar{H}_i),
\]

(6)

where \( \bar{H}_i \) is the diversity index (e.g., richness and Shannon index) at level \( i \), and \( D(H) \) is the function that converts the index into an effective number (Jost 2006). \( \bar{H} \) can be estimated in several ways depending on the diversity order (\( q \)) and the goals and assumptions of a given study. Often a simple average among subunits will be most appropriate and intuitive. Unbalanced sampling effort and some research questions might justify alternative weighting schemes (e.g., Chiu et al. 2014), but results can be difficult to interpret in the context of information theory when \( q \neq 1 \) (Jost 2006, 2007; Chao et al. 2012).

As a complementary alternative to level-wise partitioning, we propose group-wise partitioning, which summarizes diversity for each group at each hierarchical level. Instead of a single \( \alpha_i \) and \( \beta_i \), at level \( i \), we will have separate estimates for each of the \( n_{i+1} \) groups at the next level up. For example, if individuals were sampled from four populations, we would have four estimates of mean phenotypic complexity and four estimates of among-individual diversity. This approach facilitates comparisons among groups at each hierarchical level, whereas level-wise partitioning emphasizes comparisons among levels in the hierarchy. Both partitioning designs are illustrated in figure 2.

The partitioning scheme above, adopted from community ecology (Lande 1996; Veech et al. 2002; Jost 2007), is entirely general with respect to the order \( q \). Previous approaches to phenotypes or functional traits have decomposed diversity at specified levels. However, the lowest level of organization or hierarchy is usually the individual organism. In contrast, our approach decomposes phenotypic complexity at all hierarchical levels, including the within-individual level; the complexity of the individual phenotype is quantified as the diversity of phenotypic elements. The approach can be implemented for a variety of diversity indices and should not be taken as specific to the analysis of effective numbers. Proper estimation of diversity indices is an active area of development (Bonachela et al. 2008; Marcon et al. 2014; Zhang et al. 2014). Our implementation of this approach in the empirical examples below employs equal weighting and standard calculations of effective numbers.

**Empirical Examples**

We present three case studies to illustrate how this method can be applied to different questions in ecology and evolutionary biology and provide biologically meaningful interpretations. Two of these studies are from research on chemically mediated phenotypes. The first asks how damage by a specialist herbivore affects complexity and diversity of plant defenses (Fordyce and Malcolm 2000). The second asks whether changes in chemical diversity in toads can be explained as phenotypic plasticity. The third, a reanalysis of the data of Schweitzer et al. (2008), takes individual complexity into account when asking about the relative importance of tree genotype and species for asso-
associated soil microbial communities. All data for these examples are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.7vh21 (Marion et al. 2015b).

We use hierarchical bootstrapping to approximate the uncertainty around the diversity estimates (Efron 1982). In each iteration, the appropriate subgroups contained within a level are resampled, the groups within those subgroups are resampled, and so forth. Confidence intervals approximated through bootstrapping should be interpreted with some caution, as bootstrapping might underestimate uncertainty, because bootstrap replicates are not independent random samples of the population (Schenker 1985; Efron and Tibshirani 1986; Dixon et al. 1987). This might be especially relevant at lower orders of $q$, because the importance of rare aspects of multivariate phenotypes is emphasized. This is a general limitation of bootstrapping and is not unique to our approach. Although bootstrapping might not be ideal, it is frequently used for diversity analyses (Gotelli and Colwell 2010; Marcon et al. 2012), and we include it here to provide an approximation of uncertainty.

For the analyses, we used functions from the vegan (Oksanen et al. 2012) and vegetarian (Charney and Record 2012) packages in R (R Development Core Team 2014). We implemented the group-wise partitioning approach de-
sample above using the CRAN package hierDiversity (Mar- 
ion et al. 2015a). Annotated code can be found in a zip file, 
available online.

**Intertissue Induction of Cardenolides in Common 
Milkweeds (Asclepius syriaca)**

Most milkweeds (Asclepius species; Apocynaceae) employ 
a complex blend of cardiotoxic steroids called cardeno-
lides that putatively act to deter generalist consumers. How-
ever, specialist herbivores employ behavioral or physiological 
strategies to overcome or circumvent these defenses 
(Malcolm and Brower 1989; Malcolm 1992). In an observ-

erational experiment with common milkweed (A. syriaca), 
Fordyce and Malcolm (2000) examined the qualitative 
and quantitative variation in cardenolides among five plant 
tissue types (leaves, stem epidermis, cortex, vascular cylin-
der, and pith) and whether these defenses differed be-
tween plants with or without larvae of the specialist weevil, 
Rhyssomatus lineaticollis. Larval weevils specialize on milk-
weed pith tissue, which, the authors hypothesized, was a 
strategy of spatially avoiding cardenolides.

Contrary to their hypothesis, Fordyce and Malcolm 
(2000) found that the pith tissue had the second highest 
cardenolide concentration after the vascular tissue, and thus 
weevils were not spatially avoiding cardenolides. Fur-

more, they found that damaged plants had 33% lower car-
denolide concentrations overall and that there was a quali-
tative shift toward more lipophilic compounds in damaged 
plants. Because adult weevils feed on leaves before oviposi-
tion, and damaged ramets had lower cardenolide concen-
trations in total, Fordyce and Malcolm (2000) speculated that 
R. lineaticollis females might be manipulating host-plant 
chemistry for the benefit of their offspring or choosing plants 
with lower concentrations.

To address the questions of whether damaged or un-
damaged plants were more chemically complex and whether they differed in how that complexity was partitioned among 
tissues, we reanalyzed the data set using the multiplicative 
diversity partitioning methodology described above (fig. 3). 
Within individuals, damaged plants had 1–2 more com-
pounds per tissue than did undamaged plants (α). This trend 
was most pronounced in the vascular tissue and cortex 
and least apparent in the pith and epidermis, yet the chem-
ical compositions among tissues in undamaged plants 
(\(\beta = 2.11\) [95% confidence interval (CI), 2.01–2.21]) were 
more different than the tissues of plants with signs of 
weevil damage (\(\beta = 1.61\) [95% CI, 1.54–1.68]; fig. 3A). 
There was almost twice as much chemical turnover among 
tissues of undamaged plants compared with tissues from 
damaged plants (0.3 [95% CI, 0.27–0.33] vs. 0.16 [95% 
CI, 0.14–0.18] for \(q = 1\); fig. 3C).

At the among-individual within damage-treatment level, 
damaged plants had approximately one compound more 
than undamaged plants and approximately two more ef-

effective compounds overall. Interestingly, for the highest-
diversity orders, damaged plants had slightly higher β di-

versity as well (fig. 3B), although this was largely driven by 
chemical differences in vascular tissue composition. This 
suggests that damaged individuals did not differ in low-
abundance compounds but did vary in the composition of 
the few compounds of high concentration, with approxi-
mately 10% turnover in chemical phenotype among 
individuals (fig. 3D).

Fordyce and Malcolm (2000) were able to infer dif-
fences in chemistry between damaged and undamaged 
plants, but their characterization of these differences re-

mained incomplete. Using the diversity-partitioning ap-

proach, we add three biological insights. First, similarity 
among tissues within damaged plants was greater than simi-

larity among tissues within undamaged plants (fig. 3A–3C). 
Although they could not quantify it, Fordyce and Malcolm 
(2000) hypothesized that females may assess and manipulate 
plant chemistry before oviposition, narrowing the chemos-
tropic uncertainty of cardenolide composition for their 
offspring. The diversity-as-complexity approach provides 
evidence that chemical variation among tissues is lower in 
weevil-damaged plants, supporting their hypothesis. Sec-

ond, this change in complexity was accompanied by a shift 
in partitioning; among-individual variation was increased 
as damaged plants appeared to become more heteroge-
nous in chemical phenotype (figure 3B–3D). Thus, in a 
patch with ramets infected by R. lineaticollis, the associ-
ated increase in ramet chemical heterogeneity might affect 
the colonization and assembly of subsequent herbivores in 
the community. Finally, damaged plants were more chem-
ically complex in that they expressed a greater effective num-
ber of cardenolides in their tissues. The increase in the effec-
tive number of cardenolides found in damaged plants might 
explain why plants that are colonized by weevils early in the 
season experience less cumulative herbivory later (Van 
Zandt and Agrawal 2004) if later herbivores are unable to 
tolerate the increased cardenolide diversity.

**Experimental Induction of Chemical Defenses in 
American Toads (Bufo [Anaxyrus] americanus)**

Many amphibians display remarkable plasticity in their re-
sponses to environmental conditions (Newman 1992; Relyea 
2001), and the effects of natural enemy cues on larval am-

phibian plasticity have been well documented (see Benard 
2004 and Relyea 2007 for reviews). Yet, to our knowledge, 
the plasticity of amphibian chemical defenses has been 
studied only a handful of times (e.g., Benard and Fordyce 
2003; Hagman et al. 2009; Hayes et al. 2009), which is sur-
prising considering that, among vertebrates, amphibians are most notable in their diversity of putative chemical defenses (Daly 1995).

We applied our partitioning method to a subset of previously unpublished data from a study that assessed the plasticity of chemical defenses (bufadienolides) in adult American toads (B. americanus) following repeated expression of the parotoid glands. This study tested the hypothesis that toads facultatively alter the composition of their defensive secretions in response to frequent attacks. The complexity-as-diversity approach allows us to ask whether the diversity of compounds is affected by repeated expression.

Parotoid secretions were collected on preweighed filter paper after manual expression by compressing each gland between two fingers. Following an initial sampling, toads were randomly assigned to one of three expression-frequency treatments (Np42)—frequent expression (5×), medium expression (1×), or low expression (0×)—and sampled over a 5-month period. At the experiment’s conclusion, toad glands were expressed one final time for comparison, followed by high-performance liquid chromatography (HPLC) of the secretions. Here we compare the baseline chemotypes to the final chemotypes among treatments.

The traditional ordination-based hypothesis test (distance-based redundancy analysis [dbRDA]; Legendre and Anderson 1999) might support the hypothesis that parotoid expression led to statistically significant chemotypic differences among treatments (F2,35 = 3.473, P = .015; fig. 4A). However, total concentrations of bufadienolides were significantly lower in the high-expression treatment (F2,35 = 25.212, P < .001; fig. 4B), suggesting an alternative explanation. A simple depletion of bufadienolides from repeatedly expressed glands might generate differences as a side effect. As rare compounds become lost (or undetectable),

**Figure 3:** Diversity profile plots of chemotypic β diversity averaged among tissues within individual milkweed ramets (A, C) and among ramets of Asclepius syriaca with or without herbivore damage (B, D; Fordyce and Malcolm 2000). Damaged plants (dashed lines) were identified by the presence of characteristic oviposition scars produced by the specialist weevil Rhysomatus lineaticollis; undamaged plants (solid lines) lacked such scars. For diversity orders q = 0–5, chemotypic variation among tissues or ramets is expressed as the effective number of chemotypes or unique chemical blends present (A, B) or the effective compound turnover (C, D). Shaded confidence intervals for each treatment are 2 SEs. Errors for B and D were calculated via hierarchical bootstrapping, because there is only one among-plant observation for each treatment.
the distribution of relative abundances can become distorted, analogous to a bottleneck effect on genetic diversity. The diversity partitioning approach provides an intuitive way to evaluate this alternative hypothesis.

Diversity profile plots illustrate three key impacts of repeated parotoid expression on the diversity of bufadienolides. First, $\alpha$ diversity (chemical complexity of a given toad’s secretion) decreases (fig. 4C), as we might expect from depletion. Second, $\beta$ diversity increases (fig. 4D), as expected by independent random sampling (like genetic drift) as different toads lose compounds by chance. Finally, the way $\alpha$ diversity decreases and $\beta$ increases differs for different diversity orders of $q$. As with a genetic bottleneck effect (Luikart et al. 1998), rare compounds are lost rapidly (changing diversities of order $q = 0$) without dramatically shifting the relative abundances of more common compounds (diversities of higher order change less). Equivalently, in community ecology, it is generally understood that richness ($q = 0$) is much more sensitive to sampling than Shannon’s $H$ ($q = 1$) or Simpson’s $\lambda$ ($q = 2$; Colwell and Coddington 1994; Chao et al. 2005).

We further supported the depletion hypothesis by examining the impact of sampling on the ordination test. First, simply raising the detection threshold for compounds in the low-expression treatment to mimic the effects of lowered concentration (setting all compounds with
relative abundance <0.15 to zero and recalculating relative abundance of the remaining compounds) made the difference disappear ($F_{1.35} = 1.339, P = .305$). Second, we compared the original low-expression data to the recalculated low-expression data and found high statistical support for a difference ($F_{1.28} = 5.4, P = .006$).

In this example, analyzing the chemical complexity of parotoid secretions as diversity within and between toads provides a clearer view of experimentally induced changes than that offered by the more abstract results of multivariate ordination. This is because the complexity is parsed into interpretable and biologically relevant estimates. The differences illustrated in figure 4A might be taken as evidence for the idea that toads produce different chemical phenotypes as a plastic response to repeated attacks. However, borrowing an insight from population genetics (i.e., the random sampling of genetic drift) regarding the effects of depletion on diversity profiles, together with evidence of depletion in figure 4B, leads us to infer that the chemical differences between toads exposed to different treatment levels reflect random drift owing to depletion of bufadienolides in frequently expressed parotoid glands.

Plant Genotypic Variation and Soil Microbial Phenotypic Diversity

Gene expression or metabolic markers can also be used to assess phenotypic complexity and diversity in a variety of contexts. Schweitzer et al. (2008) used phospholipid fatty acid (PLFA) biomarkers to assess rhizosphere microbial community composition as a complex extended phenotype of *Populus* trees, asking whether PLFA profiles consistently differed among tree genotypes in a common garden. With 19 PLFAs assayed, they chose to summarize the PLFA phenotype using a one-dimensional nMDS ordination to provide a single number (the nMDS score) for each tree. They then performed univariate ANOVAs with the nMDS score as the dependent variable to quantify variation among genotypes. We reanalyzed a subset of their data to illustrate how diversity partitioning can offer additional insights relative to ordination-based approaches that summarize complexity with abstract multivariate scores.

The data set includes three to four clones each of four genotypes of *Populus fremontii* and five genotypes of *Populus angustifolia* (fig. A2; figs. A1–A4 available online). For ordination, we computed dissimilarities for $q = 0$ (Sorensen distance for presence-absence), $q = 1$ (Horn distance for information), and $q = 2$ (Morisita-Horn distance for probability of identity). We used permutational MANOVA on distances (Anderson 2001) and dbRDA (Legendre and Anderson 1999) to evaluate how well the dissimilarity matrices for different levels of $q$ were accounted for by clonal genotype within each species. Depending on the analysis, genotype (within species) was estimated to account for 30%–60% of the variation among trees. This is consistent with the results of Schweitzer et al. (2008).

We then used diversity partitioning to express the variation among genotypes and species in terms of the complexity of the individual PLFA profiles. This approach agrees with the distance-based ordination analyses in the sense that genotype appears to account for a large fraction of the additive $\beta$ diversity (table 1). However, $\beta$ diversity is itself only a tiny fraction relative to the complexity of each individual (fig. 5). In terms of turnover, trees of a single genotype differed from each other by 2%, and genotypes differed from other genotypes by only 1%. Moreover, nested randomization tests did not support rejecting the null hypothesis that variation ($\beta$ diversity) between trees of the same genotype is equivalent to variation between trees of different genotypes or species.

In this example, a pattern elucidated by ordination and found to be statistically significant is shown to be biologically subtle in relation to the complexity of individual samples (table 1; fig. A2). Variation within individuals dwarfs the variation among individuals, genotypes, or species (fig. 5). Ordination-based measures start at the between-individual level (dissimilarity) and entirely ignore the within-individual level of biological complexity, which our approach captures.

### Table 1: Results from alternative methods of partitioning phospholipid fatty acid complexity within and between two *Populus* species

<table>
<thead>
<tr>
<th>Variable</th>
<th>dBRDA, %</th>
<th>PerMANOVA, %</th>
<th>Level-wise, Simpson’s $\lambda$ (percentage variance explained)</th>
<th>Group-wise, effective number (turnover)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Additive</td>
<td>Multiplicative</td>
</tr>
<tr>
<td>Between species</td>
<td>7.88</td>
<td>12.11</td>
<td>$&lt;.01 (&lt;14)$</td>
<td>1.01 (0.01)</td>
</tr>
<tr>
<td>Genotypes in species</td>
<td>37.09</td>
<td>45.69</td>
<td>$&lt;.01 (&lt;49)$</td>
<td>1.03 (&lt;0.01)</td>
</tr>
<tr>
<td>Trees in genotypes</td>
<td>55.04</td>
<td>42.20</td>
<td>.01 (79)</td>
<td>1.05 (&lt;0.01)</td>
</tr>
<tr>
<td>Within trees ($\alpha$)</td>
<td>...</td>
<td>...</td>
<td>.87 (98.6)</td>
<td>8.01</td>
</tr>
</tbody>
</table>

Note: Distance-based approaches (distance-based redundancy analysis [dBRDA] and permutational MANOVA [perMANOVA]) ignore the within-tree diversity (complexity); therefore, the percentage variance explained is only over the between-tree variation. For all estimates, we show results for diversity of order $q = 2$. Under group-wise partitioning, estimates of trees within-genotypes and within-tree diversity are averages.

* Difference between *P. angustifolia* and *P. fremontii*. 

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(i.e., α diversity). Figure 5 is consistent with the idea that the structure and function of microbial communities need to be understood at a finer spatial scale (Ettema and Wardle 2002). Our analysis of this data set also emphasizes the wide range of biological phenomena that can be treated as complex phenotypes.

**Conclusion**

Virtually all phenotypes function and evolve as integrated multivariate systems that exhibit both qualitative and quantitative variation. Yet many of the most common analytical approaches typically applied to complex phenotypic data give results that reduce complexity at the cost of biological intuition. Our adoption of diversity partitioning provides a novel framework for quantifying complex traits by hierarchically separating the variation into biologically meaningful within-group and among-group components of interest. Our case studies highlight the utility of our approach by uncovering aspects of variation in the data that other complementary methods missed. Differences between damaged and undamaged milkweeds (Fordyce and Malcolm 2000) were more thoroughly characterized as differences in partitioning of chemical complexity among plant tissues. Changes in the secretions of repeatedly attacked toads were interpreted as effects of a physiological bottleneck rather than an adaptive induced response. Dissimilarity among rhizosphere communities associated with different cottonwood genotypes and species (Schweitzer et al. 2008) was shown to be small when compared with the phenotypic complexity within each sample. In all of these examples, insight was added by analyzing the complexity of individual phenotypes as an integral component of the functional ecological diversity of the study system.

The complexity-as-diversity approach can be extended to include more information about differences among constituent elements. In community ecology, functional or phylogenetic dissimilarity has been incorporated as a distance, such as in Rao’s (1982) quadratic entropy (Mouchet et al. 2010; Chiu et al. 2014). It would be straightforward to include, for example, differences in polarity between compounds in our analyses of milkweed or toad defensive chemistry. Additionally, our approach outlined here used relative amounts as opposed to absolute amounts or abundances. Recently, Chiu et al. (2014) have extended diversity partitioning to incorporate absolute abundances, which may be of more interest depending on the question. Other extensions to this approach might be possible.

The framework outlined here is particularly appropriate for analyzing complex phenotypes whose constituent variables can be intuitively conceived as relative amounts, as in the examples given above and in Iason et al. (2005). Many other phenotypes are clearly appropriate for a complexity-as-diversity approach. Diversity of cell or tissue types might have regular scaling relationships with size, abundance, and species diversity (Bell and Mooers 1997). Diversity and dissimilarity of gene expression patterns might be related to functional complexity and modularity of phenotypes. Hierarchical analysis can be used in ecological metabolomics (Sardans et al. 2011; Jones et al. 2013) to quantify the contributions of cells, organisms, populations, and so on to the physiological complexity of ecosystems. Behavioral ecologists might quantify behavioral complexity and diversity of populations and communities by analysis of time spent doing various activities or numbers of times each behavior is performed. It is less obvious why one would use this approach on something like limb morphology, although there might be value in summarizing the unevenness of relative bone lengths or the relative contributions of several skeletal elements to a given movement. We think the complexity-as-diversity approach sufficiently differs from previous analyses that it might inspire entirely new questions or hypotheses about the ecology and evolution of phenotypes.

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Literature Cited


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An adult American toad (*Bufo americanus*), one of the organisms used as a case study. Photo credit: Todd W. Pierson.