Review

# Genetics and evolution of function-valued traits: understanding environmentally responsive phenotypes

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**Many central questions in ecology and evolutionary biology require characterizing phenotypes that change with time and environmental conditions. Such traits are inherently functions, and new 'function-valued' methods use the order, spacing, and functional nature of the data typically ignored by traditional univariate and multivariate analyses. These rapidly developing methods account for the continuous change in traits of interest in response to other variables, and are superior to traditional summary-based analyses for growth trajectories, morphological shapes, and environmentally sensitive phenotypes. Here, we explain how function-valued methods make flexible use of data and lead to new biological insights. These approaches frequently offer enhanced statistical power, a natural basis of interpretation, and are applicable to many existing data sets. We also illustrate applications of function-valued methods to address ecological, evolutionary, and behavioral hypotheses, and highlight future directions.**

## Environmentally responsive traits

Most traits studied by ecologists and evolutionary biologists change in response to environmental conditions, population density, or age. For example, the vital rates at the heart of population dynamics and life-history evolution (age-specific survivorship and reproduction) show by their very names that fitness components change with age. Respiration, photosynthesis, and other fundamental ecophysiological processes respond to $C0_2$ concentrations and temperature. Phenotypically plastic traits, reaction norms, developmental trajectories, and gene expression all change with environmental conditions and age. Understanding genetic variation in these traits and how they

### Glossary

**β(x)**: selection gradient function. A mathematical function describing the direction and magnitude of directional selection on a trait as a function of the index variable.
**Basis function**: a collection or group of linearly independent functions, $f_1$, $f_2$,...$f_n$, called basis functions because they are basic building blocks: they can be combined to form new functions by multiplying by scalars and summing. For instance, $2f_1 + 5f_4$ is a new function obtained from $f_1$ and $f_4$. The linear independence of the functions means that, for example, the function $2f_1 + 5f_4$ cannot be written via any other combination of scalars and basis functions. Common basis functions for fitting function-valued traits are Legendre polynomials [11,12] and B-splines [44].
**Constant area trade-off**: a trade-off inherent to many models of environmental tolerance and thermal performance, in which the area under the curve describing how a phenotype changes with an environmental gradient is equal for all genotypes or individuals. Because of the constant area assumption, specialist–generalist trade-offs are easily modeled [67].
**Function-valued trait**: any trait that varies as a function of another continuous predictor variable. Common examples include phenotypic plasticity, reaction norms, gene expression profiles, and physiological response curves.
**G**: the genetic covariance matrix. A matrix with genetic variances for traits on the diagonal, and genetic covariances between traits on the off-diagonals.
$\mathcal{G}$: the genetic covariance function. A mathematical function describing how genetic variances for a trait changes in response to an index variable, and genetic covariances between traits at any value of the index variable.
**Index variables**: continuous variables that predict changes in function-valued traits.
**Multivariate traits**: multiple, potentially correlated traits. Measured traits can either be distinct phenotypes in the same environment (e.g., body mass and length), or the same trait expressed in alternate ecological environments (e.g., body mass in two habitats), or the same trait expressed over time (e.g., body mass at multiple ages).
**Performance curves**: curves describing how some aspect of phenotype (e.g., locomotion, growth, etc.) varies as a function of an environmental gradient. Common examples are thermal reaction norms.
**Random regression**: a form of regression where the intercepts, slopes, and coefficients are assumed to be a random sample of a population about which one wishes to generalize. In the function-valued trait context, this often means that each sire, inbred line, dam, or random effect would have its own coefficients, expressed as deviations from the population mean coefficients.
**Template function**: a mathematical or statistical function that has the appropriate shape to model performance curve, with biological interpretability of the parameters. Variation in performance curves is then modeled as variation around the common template shape, and the relative contribution of changes of biological interest is decomposed [67].
**Univariate trait**: a single phenotypic trait.

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evolve remains challenging because they are essentially functions: the traits change continuously in response to other variables. Here, we review recent advances that offer powerful and flexible analysis methods that enhance interpretation and understanding of the genetics and evolution of these 'function-valued' traits (see Glossary).

A function-valued trait is any trait that changes in response to another variable; these continuous predictor variables are often called 'index variables'. To appreciate the difference in perspective, most univariate or multivariate analyses would consider either an individual trait (size at a given age) or multiple correlated traits (size at multiple ages). By contrast, the function-valued perspective focuses on the relationship between size and age; that is, the continuous function describing how size changes with age.

Why should the ecology, evolution, and genetics of functions, when even single traits can pose difficult challenges? First, many recurring debates in evolutionary ecology concern characterizing the amount of genetic variation in different types of trait (e.g., life history vs other traits [1–3]) and the relative strength of evolutionary constraints [4–8]. Analyzing functions gives enhanced capacity for quantifying genetic variation and detecting genetic constraints. Second, the simplifications imposed by univariate and multivariate analysis distort and obscure the nature of variation and its consequences. Much as a correlation between two traits can reveal a constraint not apparent from each trait in isolation, function-valued analyses can uncover constraints not apparent from univariate or multivariate analysis. Third, function-valued methods offer enhanced statistical power. Finally, adopting the perspective that traits are functions allows characterization of the full range of environmentally responsive phenotypes.

To illustrate, consider Figure 1, which shows univariate, multivariate, and function-valued approaches to analyzing genetic variation in size of salamanders [9]. In amphibians, size at metamorphosis often influences fitness components (survival and mating). In the univariate case, the genetic variance in size at a given age (Figure 1a) gives no information about its covariance with size at other ages. In a multivariate analysis, genetic covariances of size at multiple ages are estimated (**G**, Figure 1b), but this does not take advantage of the fact that correlations between similar ages will be larger than those between widely separated ages. By contrast, the function-valued analysis (Figure 1c), views size measurements as observations of underlying continuous growth curves (Figure 1c, left panel), and explicitly takes advantage of the fact that measurements at similar ages are more closely related than are measurements at widely separated ages. By taking advantage of the temporal ordering and continuity of growth, one can estimate a continuous genetic covariance function, $\mathcal{G}$, that portrays genetic variation in size, and covariances in size between every pair of ages, across the entire growth period (Figure 1c, right panel). The function-valued perspective offers enhanced statistical power, greater ability to detect genetic constraints, and improved understanding of phenotypic and genetic variation in environmentally sensitive traits.

## Statistical efficiency, flexibility, and enhanced power

Treating traits as functions (and measurements as information about a single underlying biological curve) affords many practical and statistical advantages. Consider a comparison of two growth trajectories (Figure 2). Different numbers of measurements exist for each individual at different ages. Function-valued approaches use these data to estimate and compare curves, despite the sampling differences (a key advantage in ecological or evolutionary studies where data may be taken at different time points, or different degrees of missing data are likely). By contrast, multivariate methods require measurements at comparable ages, necessitating insults to the data, such as binning or eliminating measurements, or ad-hoc imputations to common ages.

Curve fitting also provides a natural way to smooth noisy data and extract underlying structure or patterns in which the number of phenotypic measurements per individual is large. Data of this form are especially common for environmental monitoring from data loggers and transcriptomic analyses of gene expression.

Function-valued statistical methods can be more powerful and robust than with multivariate methods. Griswold *et al.* [10] showed that even when individuals are measured at identical index values, multivariate analyses have less power than even basic function-valued methods. As the number of measurements per individual increases, multivariate methods lose power, whereas the power of function-valued methods remains stable or even improves. The reason is function-valued methods use information about the continuity of the underlying function that is ignored by multivariate methods [10].

Griswold *et al.*'s findings [10] have two important implications. First, function-valued approaches could yield more insight and power from currently used experimental designs, with existing data. Second, function-valued methods have statistical advantages even when the curves estimated are purely phenotypic, from unrelated individuals: there is no need for a breeding design or pedigreed population to obtain the statistical advantages of function-valued analyses. The power advantages are apparent even when there is no hierarchical quantitative genetic design, or need to predict evolutionary responses to selection and, as such, the function-valued approach is also well suited to areas outside of evolutionary quantitative genetics. Function-valued analyses potentially offer greater power and insight into areas of ecology, genomics, and animal behavior, where purely phenotypic data are typical.

## Selection response and genetic constraints

A crucial goal for evolutionary biologists and breeders is to predict how function-valued traits evolve. For predicting evolutionary responses, the ability of function-valued methods to use information about the order and spacing of measurements leads to substantially improved statistical and predictive power [11,12].

The function-valued framework for projecting evolution is based on the standard model for quantitative traits [13]: the phenotype of an individual (e.g., size) at each index value (e.g., age) is the sum of additive-genetic and non-genetic (environmental) components that are normally distributed. The evolutionary change over one generation

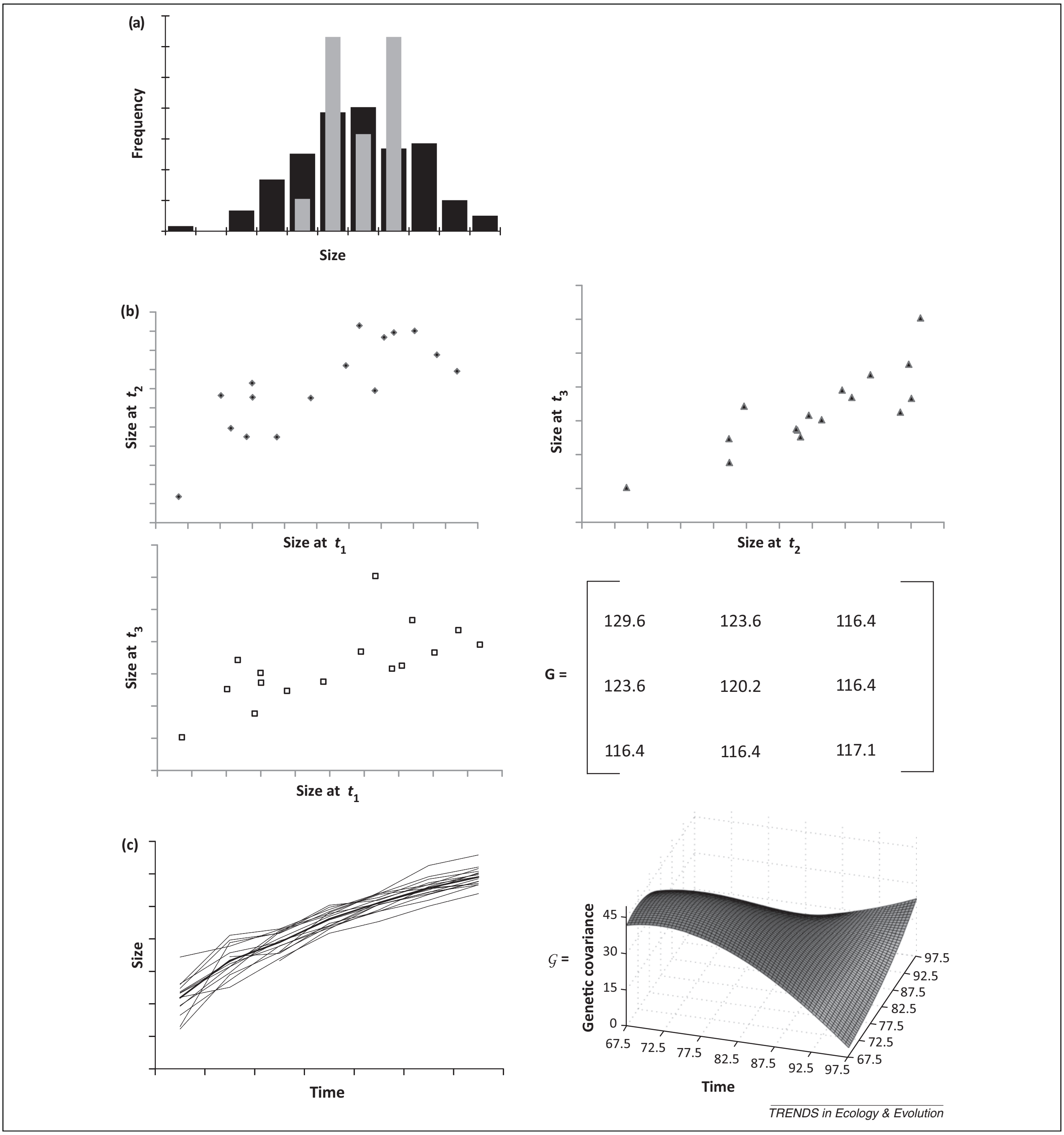

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**Figure 1**. Three representations of genetic variation for size in salamanders. The data are from three ages: 83%, 88%, and 93% of the larval period. Univariate **(a)**: the size distributions for individuals (black) and the means of full sib families (gray) at Age 1. Multivariate **(b)**: scatter-plots showing the bivariate distributions of family means for size at three ages (83%, 88%, and 93% of the larval period, $t_1$, $t_2$, and $t_3$). The estimated genetic covariance matrix is at the lower right. Function-valued **(c)**: size as a continuous function of age. At left, the family means are shown in broken lines, and the population mean in black. At right, the estimated genetic covariance function. Data replotted from [9].

in the mean value of the trait at index value $a$ is as follows (Equation 1):

$$\Delta\bar{z}(a) = \int_{a_{\min}}^{a_{\max}} \mathcal{G}(a, x)\beta(x)dx \qquad [1]$$

Equation 1, derived by Kirkpatrick and Heckman [11] (also see [12,14]), shows that evolutionary responses depend on the additive-genetic covariance function, $\mathcal{G}$, and the selection gradient function, $\beta(x)$. The value of $\mathcal{G}(a, a)$ gives the additive-genetic variance for the trait at index value $a$, and $\mathcal{G}(a_1, a_2)$ gives the genetic covariance between the traits at index values $a_1$ and $a_2$. $\mathcal{G}$ can be evaluated at any index values (within the range of the data), regardless of whether data were taken at those values. The function $\beta(x)$ describes the strength of directional selection favoring

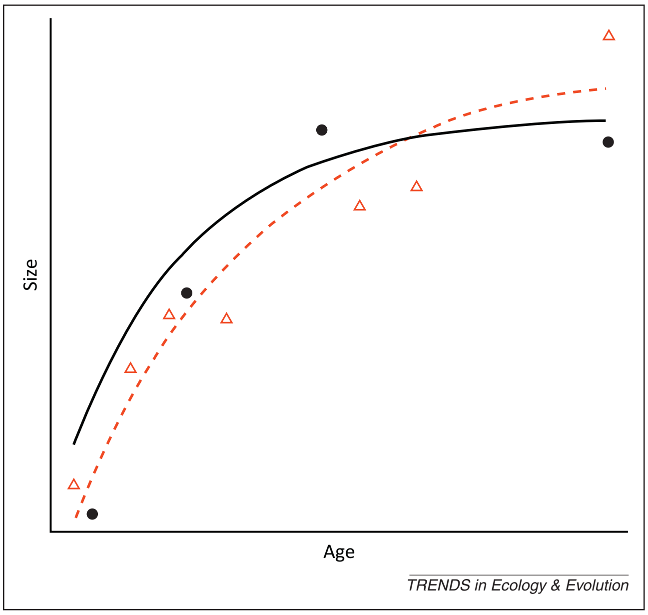

**Figure 2**. Measurements made at differing ages (filled symbols) and underlying growth curves for two individuals.

an increase (if positive) or decrease (if negative) in the trait at index value $x$. For example, for size as a function of age, $\beta(x)$ indicates selection favoring increased or decreased size at different ages, such as the age of first reproduction [15]. Similarly, for thermal performance traits, $\beta(x)$ indicates selection favoring increased or decreased performance at different temperatures [16,17].

Function-valued methods also allow a more precise evaluation of evolutionary constraints, through the estimation of the eigenvalues and eigenfunctions (the function-valued equivalent of a principal component analysis) of $\mathcal{G}$. Box 1 shows the eigenfunctions of $\mathcal{G}$ for the salamander population from Figure 1. Eigenfunctions identify changes in the function-valued trait for which there is abundant genetic variation, and those lacking genetic variation. The changes for which there is very little genetic variation are said to be in the nearly null space of $\mathcal{G}$: a region of phenotypic space that is off limits to evolution because of a lack of genetic variance. The nearly null space is the function-valued equivalent of the evolutionary lines of greatest resistance [8]; the leading eigenfunctions are the function-valued equivalents of evolutionary lines of least resistance [18,19]

An additional statistical advantage of eigenanalysis of function-valued traits is that it can be used to reduce the number of parameters estimated: in some cases, the first few eigenfunctions explain most of the genetic variation in a function. By estimating the leading eigenfunctions, one gains improved statistical power and accuracy in estimating both selection responses and the nearly null space [20,21]. An important question is when low-dimensional representations of high-dimensional phenotypes can fully capture relevant evolutionary properties, such as the ability to respond to selection. Although studies of function-valued traits suggest that most genetic variance typically exists in fewer dimensions than the number of independent 'traits' investigators perceive and measure (e.g., [22,23]), results from studies of traditional multivariate traits are mixed [24–26]. More genetic studies on a wider variety of function-valued traits, along with artificial selection experiments, will clarify whether some dimensions of phenotypes and function-valued traits are lacking genetic variance and unable to respond to selection.

### Box 1. Eigenfunctions of a genetic covariance function

The function-valued perspective quantifies patterns of variation that can lead to a response to selection with the genetic covariance function, $\mathcal{G}$. Figure 1c (main text) shows this function for the salamander data [9]. The estimated covariance function is not restricted to a particular form, except for a few mild conditions, including having non-negative variances, correlations in the range (–1, 1), and eigenvalues $\geq 0$. Although the covariance function gives a complete description of genetic variation in the population, there is an alternative way to view the data that often facilitates understanding. The covariance function can be decomposed via principal components analysis as (Equation I):

$$\mathcal{G}(x,y)=\sum_{i=1}^{\infty}\lambda_i\varphi_i(x)\varphi_i(y) \qquad [\mathrm{I}]$$

In Equation I, the $i$-th eigenfunction, $\varphi_i$, is the function-valued equivalent of an eigenvector, and represents a direction of genetic variation [22]. The corresponding eigenvalue $\lambda_i$ quantifies the amount of genetic variation explained by that direction. The eigenfuntions, $\varphi_i$, represent statistically independent avenues along which a function-valued trait can evolve [22]. In practice, one tends to consider only the most important directions of variation, that is, only the $\varphi_i$s corresponding to the largest $\lambda_i$s. Figure I shows PC1 and PC2; that is, $\varphi_1$, (91% of genetic variation) and $\varphi_2$ (8.9% of genetic variation) for the salamander data.

Selection can cause rapid changes in the space of the PCs with large amounts of genetic variation. Figure I shows that there is abundant variation for increasing or decreasing size at all ages: $\varphi_1$ explains most of the genetic variance, and is always above the x-axis, indicating positive genetic covariance in size across ages. By contrast, there is much less variation for $\varphi_2$, which describes changes that have opposing effects on size at early and late ages, illustrated by $\varphi_2$ crossing the x-axis. The selection response will be very slow (or zero) for changes corresponding to eigenfunctions with little (or no) variation. In these salamanders, there is almost no measurable variation for more complicated deformations of the growth trajectory, for example increasing size at early and late ages and decreasing size at intermediate ages.

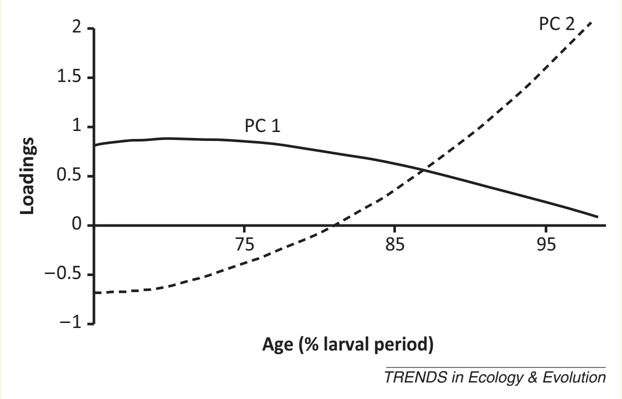

**Figure I.** Eigenfunctions for the genetic variance–covariance functions for salamander growth. Reproduced, with permission, [9].

Working with covariance functions also allows better estimation of breeding values, an important parameter in both basic and applied contexts [13]. In applied settings, many traits under artificial selection in domesticated animals vary with physiological status: for instance, milk production is a function of the number of days since calving. Cattle breeders quickly appreciated the merits of function-valued analyses, and most large dairy breeding programs now use them (via random regression) to determine the genetic value of individuals and herds [27,28]. A consistent result has been that function-valued methods provide more accurate estimates of breeding values than do traditional multivariate methods [28,29].

## Implementation methods: an overview

### *Template functions and 'parameters as data'*

Given the advantages of function-valued approaches, what implementation strategies exist? In general, three approaches are possible. One approach is to analyze variation around a template function with the desired shape or biological interpretation (Box 2). Another is to assume a specific parametric form of the curve for each individual or genotype, and estimate the curve parameters of each individual or genotype. One then uses these parameters as 'data' for subsequent analyses of patterns of genetic variation, natural selection, or treatment comparisons (e.g., [30]). Studies such as this, for example, have characterized reaction norms [31,32] and tolerance to herbivory [33,34] for genotypes with linear or quadratic regression. The 'parameters as data' approach requires each individual or genotype to have sufficient data, and makes the restrictive assumption that all individuals or genotypes are fully characterized by the chosen parametric model. The method is also inefficient: each curve is fitted independently, meaning that information from the entire data set is not utilized simultaneously. An additional complication, rarely addressed, is that regressions for individuals and genotypes are estimated with error, and properly accounting for these errors through subsequent analyses can be problematic (but see [30,35]).

### *Mixed models and random regression*

In evolutionary studies, an alternative is to use a mixed-model framework to model variation in individual curves around a mean trajectory, while accounting for genetic relationships between individuals. The methods are closely related to other applications of hierarchical modeling, including individual growth curve analysis [36]. Several

### Box 2. Decomposing modes of variation

For many function-valued traits, there are biological constraints on the possible shapes of curves and variation in them. A benefit to analyzing curves rather than measurements is that these constraints can be used to design biologically motivated hypothesis tests.

One approach, known as template mode variation (TMV) [67], assumes a common template shape or function for all individuals and genotypes. Thermal reaction norms for biological rates (performance curves) are typically non-negative functions with a single, intermediate maximum. Deviations from the mean template curve for each individual represent phenotypic and genetic variation of interest.

The TMV approach tests specific *a priori* hypotheses about the modes of variation in performance curves (Figure I) [67]. A vertical shift in the curve implies differences in overall performance; if performance is closely linked to fitness, individuals with a positive vertical shift (above the dashed line) would have higher fitness under all conditions. Both the horizontal shift and the specialist–generalist modes illustrate a constant area trade-off [94], where increases in performance under any specific condition decrease performance elsewhere. These three modes of variation can be characterized by a three-parameter model that quantifies variation around the template curve [67]. One of the strengths of the TMV method is that it partitions the variance in parallel to these specific hypotheses. The constant-area assumption is inherent to the TMV model, and allows tests of hypotheses of interest about trade-offs, but may not be applicable to scenarios lacking explicit biological hypotheses about trade-offs.

One recent study applied TMV to thermal performance curves in *Drosophila serrata* [90]. Isofemale lines were established from three populations; locomotor activity was measured on males and females at seven different temperatures. The TMV model explained from 12% to 34% of the variance, with more variation explained in females than in males. No sex or population exhibited more than 1% of the variance in vertical shift. The specialist–generalist mode explained the most variance (7–28%), followed by the horizontal shift (1–11%). These data suggest that rather than good 'all purpose' genotypes, or particular temperature specialists, most genetic variation in thermal performance was of the 'Jack of all trades is the master of none' variety. The major limitation at this time is that methods for comparing two or more groups are not yet available.

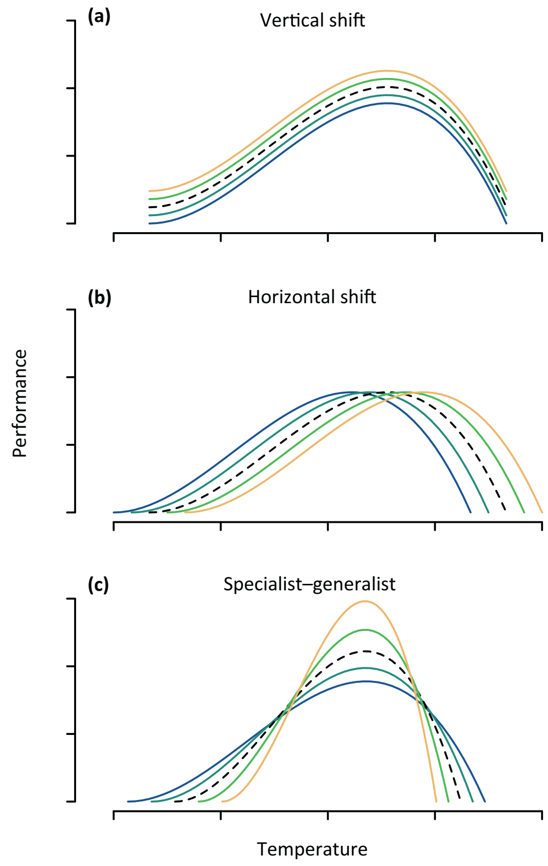

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**Figure I.** In template mode variation analyses [67], variation is statistically partitioned among three components: vertical shift, specialist–generalist, and horizontal shift.

software packages have this capacity, including SAS [37], WOMBAT [38], ASREML [39], and R [40]. When fitting functions, it is useful to write them as a weighted sum of curves of known shape (basis functions); the set of curves can be simple, yielding linear or multiple regressions, or they can be more flexible, yielding, for example, B-splines or Legendre polynomials. The coefficients of these curves are viewed as random variables, varying from individual to individual. The regression coefficients and overall population parameters can be estimated by restricted maximum likelihood and other linear mixed model techniques [41]. By making use of individuals of known relatedness, variation in the regression coefficients can be partitioned into genetic and environmental deviations. The resulting estimates of genetic and environmental covariance matrices of the random regression coefficients can then be used to calculate genetic and environmental covariance functions. Variation attributable to random effects (typically sires, although other quantitative genetic units are possible) are used to estimate $\mathcal{G}$. Importantly, $\mathcal{G}$ describes patterns of genetic covariation in the focal trait, exactly analogous to $\mathbf{G}$, except that it is a function of the index variable. $\mathcal{G}$ can be evaluated at specific values of the index variable to present $\mathbf{G}$.

The random regression approach has several advantages: (i) the linear mixed model, REML framework facilitates statistical inference and hypothesis testing; (ii) by using a function that is of lower order than the number of observations per individual, fewer parameters are estimated, resulting in enhanced power and accuracy; (iii) breeding values for individuals can be estimated at any point along the index variable; and (iv) it can also be used to analyze phenotypic covariance functions [42].

The random regression approach, when implemented with polynomials, has potential limitations worth noting. First, higher-order polynomials are 'wiggly' [43], with curves sometimes exhibiting dramatic oscillations. Cubic or higher-order polynomials can suffer from 'end of range' problems: errors associated with estimation at the extremes of the data can be severe, especially when there is imbalance in the number of records at each end of the range [44,45]. These problems can usually be detected by comparing $\mathcal{G}$ when evaluated at the end of the range of the data to a multivariate estimate of $\mathbf{G}$ (e.g., [43]); they can also be mitigated by using one type of basis function (e.g., splines) to model the mean trajectory, and a second type of basis function (e.g., polynomials) to model the individual differences from the mean [44]. Judicious model fit is essential; a common approach is to start with simple models, increase the order of fit, and use likelihood ratio tests (or information criteria) to evaluate when the model likelihood does not improve significantly. It is important to include appropriate fixed effects (block, year, etc. [44]), and evaluate model fit of both odd- and even-ordered polynomials. Collectively, these considerations suggest that for any study, the sensitivity of the conclusions to the choice of basis function, model fit, and mode of analysis should be evaluated.

## Case studies

Function-valued methods have been applied to numerous ecological and evolutionary topics: a representative sample (Table 1) includes diverse organisms (flies, humans, free-living mammals, and birds), traits (gene expression, thermal performance, aging, allometry, and phenology), and techniques (manipulative experiments and long-term monitoring). We highlight studies illustrating promising applications.

### *Sexual selection and behavioral preference functions*

Female preference functions for traits of potential mates are important for understanding the evolution of mating behavior and the form and strength of sexual selection [46,47]. Ritchie and colleagues have examined female mating preference as a function of song syllable number in katydids [46] and song frequency in fruit flies [47] to test alternate models of sexual selection. Because female mating preferences vary as a function of male traits and their social environment, they are readily analyzed as function-valued traits in the same manner as 'typical' plastic traits or reaction norms that change in response to environmental or ecological conditions [48,49].

McGuigan *et al.* [49] used a novel application of function-valued analyses to examine genetic variation in female preferences for multiple male traits. In their experimental design, female flies generated via a half-sib mating design were given a choice between mating with two male flies: a focal fly and another from the laboratory population. Focal male flies were scored for mating success (successful or unsuccessful), and for nine continuously varying cuticular hydrocarbon (CHC) traits. Because the females came from a mating design, McGuigan *et al.* [49] used random regression to estimate genetic variation and covariation in female preferences for the nine male CHC traits; male CHC traits were the index variables in the random regression. The first principal component (PC) of the female preference function explained 64% of the genetic variation, suggesting high genetic correlations among female preferences for different male traits [49]. We expect that similar efforts describing mating preference functions can be profitably applied to other studies of sexual selection.

The functional approach exemplified by these studies [46–49] can also be applied to other studies of behavioral preferences. Herbivore preference functions for different host plants or defensive chemicals could be constructed in a manner similar to constructing female preference functions for male phenotypes. Likewise, in systems where foraging or behavioral decisions are made in response to multiple factors, the covariance between preferences to multiple stimuli can be evaluated with random regression. Application of function-valued methods in a quantitative genetic framework would facilitate testing hypotheses about evolutionary potential and constraint in preference functions.

### *Environmental sensitivity of genetic correlations*

The long-term studies of feral Soay sheep (*Ovis aries*) illustrate how novel function-valued methods can be applied to long-standing questions. Intensive monitoring of the population has resulted in a detailed pedigree, along with morphological and life-history measurements. Robinson *et al.* [50] applied random regression models to examine phenotypic, genetic, and environmental correlations

**Table 1. Exemplar studies applying function-valued methods to test ecological and evolutionary hypotheses**

| Index variable | Focal variable | Species | Approach[a] | Refs |
|---|---|---|---|---|
| Age | Growth | Finches (*Fringilla coelebs, Carduelis chloris*, and *Carpodacus erythrinus*) | LP | [68] |
| | Growth | Blue tit (*Parus caeruleus*) | LP | [69] |
| | Annual fitness | Flycatchers (*Ficedula albicollis*) | RRAM, LP | [70] |
| | Wheel running behavior | House mice (*Mus domesticus*) | RM-ANOVA | [71] |
| | Early-life fecundity and aging | Red deer (*Cervus elaphus*) | RRAM | [72] |
| | Early and late fecundity | Humans (*Homo sapiens*) | RRAM, LP | [73] |
| | Transcriptome | Fruit fly (*Drosophila melanogaster*) | CP | [74] |
| | Size | Bighorn sheep (*Ovis canadensis*) | RRAM, LP | [75] |
| | Age-specific reproduction | Soay sheep (*Ovis aries*) and red deer (*C. elaphus*) | RR | [76] |
| | Weight and leg length | Soay sheep (*O. aries*) | RRAM, LP | [77] |
| CHC | Mating preference in two environments | Fruit fly (*Drosophila serrata*) | RR | [78] |
| | Mate choice | Fruit fly (*Drosophila bunnanda*) | RR | [49] |
| | Mating success | Fruit fly (*D. serrata*) | RR | [79] |
| Condition | Mating success | Fruit fly (*D. bunnanda*) | RR | [80] |
| Environmental quality (survivorship of lambs) | Horn length, body weight, and parasite load | Soay sheep (*O. aries*) | RRAM, LP | [50] |
| | Birth weight | Soay sheep (*O. aries*) | RRAM, LP | [53] |
| Heat resistance | High temperature stress | Fruit fly (*D. serrata*) | Artificial selection | [81] |
| Size | Allometry | Threespine stickleback (*Gasterosteus aculeatus)* | RR | [82] |
| Temperature | Egg laying | Common gull (*Larus canus*) | RRAM, | [83] |
| | Growth rate | Snails (*Potamopyrgus antipodarum*) | TMV | [60] |
| | Locomotor performance | Wasps (*Aphidius ervi*) | LP | [84] |
| | Egg laying and clutch size | Great tit (*Parus major*) | RRAM, LP | [85] |
| | Breeding timing | Great tit (*P. major*) | RRAM, LP | [61] |
| | Growth rate | Cabbage white (*Pieris rapae*) | TMV | [86] |
| | Growth rates | Cabbage white (*P. rapae*) | RR, LP | [87] |
| | Growth rate | Bacteriophage (G4) | TMV | [88,89] |
| | Locomotor activity | Fruit fly (*D. serrata*) | TMV | [90] |
| | Growth | Coho salmon (*Oncorhynchus kisutch*) | LP | [91] |
| | Timing of reproduction | Great tit (*P. major*) | RRAM | [92] |
| Temperature and age | Growth rate and wheel running | Cabbage white (*P. rapae*) and house mice (*M. domesticus*) | LP | [67] |
| Time | Speech and sound | Humans (*H. sapiens*) | Phylogeny | [93] |

[a] Abbreviations: CP, Character Process model [43,74]; LP, Legendre polynomials fit to covariance matrix; RR, random regression; RM-ANOVA, repeated measures ANOVA; RRAM, random regression, animal model; RR, LP, random regression on Legendre polynomials; TMV, template mode of variation.

between horn length, body weight, and parasite load as functions of environmental quality. Environmental quality was estimated as the fraction of lambs that survived the year, providing a continuous index variable reflecting good and bad years for sheep survival. The authors found that genetic correlations among ecologically important traits in a wild population were weaker under benign environmental conditions, as predicted by laboratory-studies [51] and theory [52].

The approach used by Robinson *et al.* [50] (also see [53]) suggests promising avenues for future research. Defining environmental quality in a way that integrates the abiotic and biotic consequences for the organism leads to a composite measure of good and poor environments in a relevant 'currency'. Their approach, which is reminiscent of the phytometer method of using the performance of tester plants to measure environmental quality [54–56], suggests a new means of assaying how environmental quality affects variation and covariation in ecologically important traits. Their approach holds great promise for systems not amenable to experimental manipulation, or to field or common garden experiments where the relevant biotic and abiotic factors are unknown.

## Limitations

Although the logic of treating traits as functions is compelling, there are two challenges to routine adoption of function-valued approaches: the cost of additional measurements (both obtaining and analyzing them) and unfamiliarity with function-valued analyses.

To use function-valued approaches, multiple measurements per individual or genotype are required, which for a fixed effort or cost, conflicts with maximizing the total number of individuals and genotypes; this limitation applies equally to multivariate approaches. In some cases, the quality of the additional information gained can make up for reduced sample sizes. Behavioral data are commonly gathered as counts of discrete acts captured on videotape. It requires only a change in perspective to record the time of each act, as well as their total number.

For all the practical difficulties in gathering function-valued data, perhaps a bigger hurdle is a lack of knowledge. Even if the potential advantages are clear, mastering the necessary statistical machinery and software can be challenging. The task of choosing a basis function falls outside the education of many biologists. The relevant literature is highly technical. Many advances are presented in the statistics or animal breeding literature, which are not widely read by ecologists or evolutionists; there is no textbook on function-valued analyses in ecology and evolution (but see [57–59] for a start). Although function-valued trait analyses can be implemented in many software packages, user-accessibility could often be improved.

## Prospects

Functional analyses have been instrumental in quantifying patterns of genetic variation and evolution for thermal performance curves. To our knowledge, function-valued methods have not been applied to other types of nonlinear, continuous reaction norm, despite a substantial literature on reaction norm variation and evolution. There are excellent opportunities to evaluate whether the lessons learned from functional analyses of temperature apply to other types of phenotypic plasticity.

Function-valued methods are ideally suited for addressing key questions in how organisms will cope with global

### Box 3. Function-valued phenogenomics

Geneticists are increasingly able to manipulate gene expression to observe phenotypic effects. These experiments can be a powerful tool for understanding the genotype–phenotype map critical to evolutionary inferences about the nature of selection on the genome. Such experiments yield a response to genetic manipulation that is actually an entire function. A challenge is to take advantage of the function-valued nature of the data during analysis.

The level of expression of Sonic hedgehog (SHH) in the developing vertebrate brain is a candidate process generating variation in the upper jaw and face. Young *et al.* [95] manipulated SHH in chicken embryos by injecting embryos with SHH antibody expressing cells to knock expression down, or with SHH protein to increase expression, resulting in treatments that ranged from a near absence of SHH expression (on the left of Figure Ia), to a doubling over normal levels (on the right of Figure Ia). Embryos were scanned by microcomputed tomography, and the three-dimensional surfaces of the embryonic head constructed. A network of 67 recognizable landmarks was analyzed using geometric morphometric methods, a family of multivariate techniques that respect the spatial locations of the sampled points, and approximates the functional surfaces of interest. The results clearly show the quantitative sensitivity of morphology to the SHH dose in the developing forebrain (Figure Ib), supporting the idea that variation somewhere in the SHH signaling cascade can account for the variation in facial width, for example in humans, where the width of the face is a major axis of variation among phenotypically normal individuals. An important challenge and goal is to analyze the entire set of surfaces recovered, not just the landmarks.

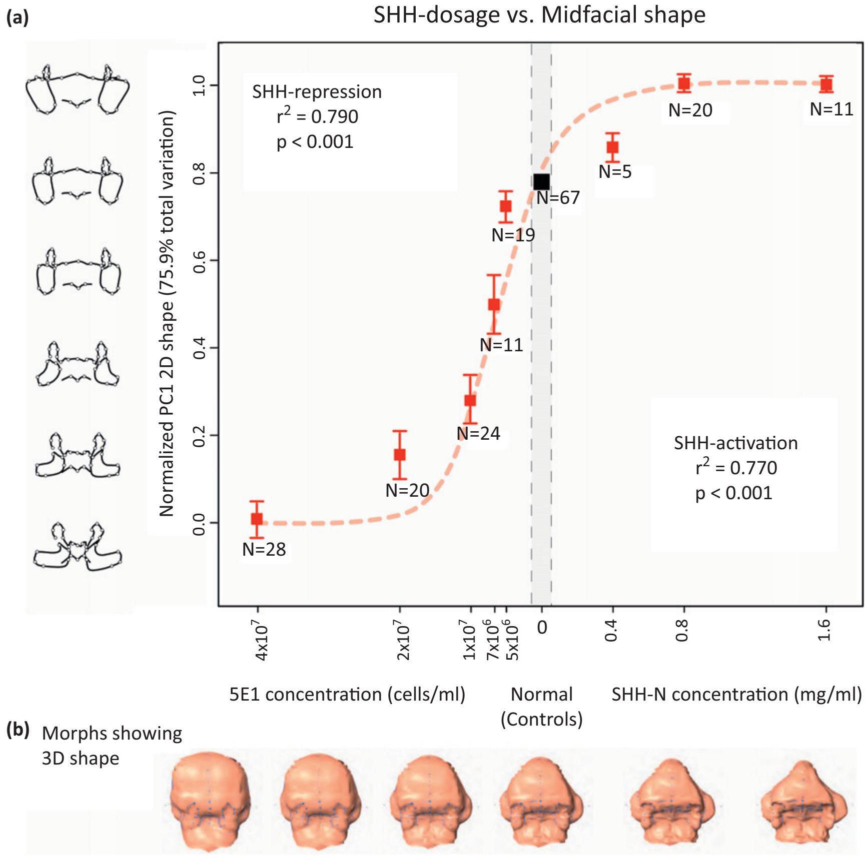

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**Figure I.** The relationship between sonic-hedgehog dosage and aspects of morphological shape. Modified, with permission, from [95].

climate change [60,61]. Climate change is altering several key index variables (temperature, seawater pH, and salinity), and function-valued methods are ideally suited for estimating genetic variation in organismal performance across these continuous gradients, and the potential for genetic constraints.

An additional challenge to the functional view of phenotypes that we have advocated is the combined analysis of multiple functions: many common phenotypes are multifunctional. To understand development, it is not enough to characterize the spatiotemporal pattern of gene expression; one must integrate it with, among other things, the pattern and direction of cellular proliferation, which is itself a function of space and time. Quantitative characterization of the genotype–phenotype map can be thought of as a complex function-valued problem. Ecologically, the distribution and abundance of species is clearly multifunctional: variables such as intra- and interspecific density, temperature, disturbance regime, and the availability of light, water, and nutrients operate together, frequently co-vary, and all affect the expression of phenotypic and genetic variation of populations.

Fortunately, increasing efforts to devise automated measurement techniques are resulting in high-dimensional functional data (Box 3). An automated analysis of behavior of groups of organisms gives rise to extensive time series of individual behavior [62,63]. Falling costs of transcriptomic analyses will make time courses of expression of tens of thousands of genes routine. Image analysis allows temporal characterization of development at the cellular level. Detailed characterization of multiple ecological variables is more feasible given automated monitoring equipment. Although challenges remain (Box 4), as inherently functional data become more common, functional analyses become necessary, rather than just a promising idea.

Efforts to take advantage of the full information in biological functions will be of particular value to integrative studies of ecology and evolutionary genetics. The usually abundant natural variation in phenotypes within and among populations constantly generates experiments that provide one with the ability to disentangle causes from correlations [64]. With a sufficiently accurate representation of phenotypes, it will be possible to determine which aspects actually cause differences in performance and fitness [65]. Such detailed information can suggest ecological mechanisms underlying those differences. Similarly, pairing the detailed phenotypic targets of selection with their genetic basis connects the indirect genomic signatures of selection with the actual forces causing evolution [66].

### Box 4. Outstanding questions

Several outstanding questions remain, including:

- Sample size requirements for evolutionary studies. Much of the work describing the superiority of the function-valued perspective come either from simulation studies [11] or comparisons of mean phenotypes [10]; recent studies of sampling strategies focus on interindividual (rather than intergenotype) ontogenetic trajectories [96]. Agricultural studies can suggest an upper bound, because sample sizes there typically dwarf even the most ambitious evolutionary genetic studies. Even rough guidelines on the number of quantitative genetic units (sires and inbred lines), individuals, and measurements per individual remain elusive.
- Working with sparse data. For phenotypic analyses, a major thrust of investigation has been developing methods that are not challenged by working with sparse, irregularly spaced, longitudinal data [97]. Extension to hierarchical designs that would permit estimation of genetic variation in sparse curves would be highly useful.
- Curve registration. An important practical challenge posed by function-valued methods is to ensure comparability among curves in individual-based analyses. Comparability is often achieved by registration of observations to a common coordinate system, that is, matching features common across individual observations. Growth curves, for instance, could be alternatively matched by chronological age, developmental stage, ontogenetic events, such as growth peak or time at growth onset, or function-specific parameters (asymptotic values, inflection points, etc.). When dealing with multivariate data, registration is facilitated by the fact that data are collected at the same value of the index variable. In the function-valued context, multiple criteria are often sensible ways to establish comparability across curves, and they might conflict with one another, for example when the number of developmental stages varies despite total development time remaining approximately constant [98]. Registration decisions should be made carefully, as they can affect the distribution of variation among curves.
- Object-oriented data. An area that might have utility in evolution and ecology is objected oriented data [99] where the functions that underlie the analyses discussed here become more complicated entities, such as shapes (Box 3), images without clear landmarks, or tree-structured objects, such as blood vessel or veination patterns. Characterizing the population mean and patterns of variation for object-oriented data is a developing area, although ecological and evolutionary applications remain rare.
- Bayesian inference. Recently developed Bayesian methods have facilitated hypothesis testing for univariate and multivariate quantitative genetic parameters (e.g., genetic variances and covariances [40]). Adoption of these methods to function-valued approaches in ecology and evolutionary biology would facilitate hypothesis testing.

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