

Reciprocal Selection at the Phenotypic Interface of Coevolution¹

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SYNOPSIS. Coevolutionary interactions depend upon a phenotypic interface of traits in each species that mediate the outcome of interactions among individuals. These phenotypic interfaces usually involve performance traits, such as locomotion or resistance to toxins, that comprise an integrated suite of physiological, morphological and behavioral traits. The reciprocal selection from species interactions may act directly on performance, but it is ultimately the evolution of these underlying components that shape the patterns of coevolutionary adaptation in performance. Bridging the macroevolutionary patterns of coevolution to the ecological processes that build them therefore requires a way to dissect the phenotypic interface of coevolution and determine how specific components of performance in one species exert selection on complementary components of performance in a second species. We present an approach for analyzing the strength of selection in a coevolutionary interaction where individuals interact at random, and for identifying which component traits of the phenotypic interface are critical to mediating coevolution. The approach is illustrated with data from a predator-prey arms race between garter snakes and newts that operates through the interface of tetrodotoxin (TTX) and resistance to it.

INTRODUCTION

Because performance traits are operationally defined in terms of meeting some organismal-level challenge, they are typically considered to exist on the “front-line” of selection (Arnold, 1983; Garland *et al.*, 1990; Garland and Carter, 1994). At first glance, then, performance traits would be expected to evolve relatively quickly because of the strength of selection they experience. However, performance traits are complex characters that comprise a variety of underlying components (Arnold, 1983). Any performance trait, such as prey-handling time or thermal tolerance, is the culmination of a variety of physiological, behavioral and morphological factors interacting within an individual. Although selection may target performance directly, it is through the evolution of these underlying component traits that performance itself evolves (Arnold, 1983; Geffeney *et al.*, 2002).

Understanding the evolution of performance therefore requires a truly integrative approach to dissecting the mechanistic relationships among component traits and among these components and performance itself. The heuristic and statistical model described by Arnold (1983) to reveal these relationships has taken us a long way toward understanding how selection is filtered through performance to influence the evolution of underlying traits. This path-based approach remains one of the more powerful tools in integrative biology and continued refinements of the tool have increased the breadth of problems and types of interactions among traits that can be considered under its mantle (Arnold, 1988; Kingsolver and Schemske, 1991; Scheiner *et al.*, 2000).

Nonetheless, the application of the trait-performance-fitness paradigm remains primarily the realm of ecological and evolutionary physiology. As a result, performance traits are typically considered in the context of interactions between individual organisms and their environments. Many of the most critical challenges, and consequently the strongest selection, that an organism meets come from ecological interactions with other species. When a performance trait exists with respect to another species, the selective environment of importance might itself be a performance trait made up of other underlying characters. Such is the case for coevolutionary interactions.

Coevolution is driven by reciprocal selection that results from ecological interactions among individuals of two or more species (Thompson, 1994). Such reciprocal selection occurs at a phenotypic interface that comprises the trait (or traits) that determine fitness outcomes of the interaction (Brodie and Brodie, 1999b). The phenotypic interface of coevolution will almost invariably involve complementary performance traits, because the critical aspect of any species interaction is how well one species “performs” against the other (Fig. 1). For example, in a plant-herbivore interaction, the phenotypic interface might involve the induction of chemical defenses by the plant and the resistance to those same chemicals by the herbivore. The plant performance trait would include not only baseline levels of defensive compounds, but also mechanisms of recognition of herbivory and biochemical regulation of defensive compound production. Herbivore performance could include both physiological and behavioral components of resistance to the effects of defensive compounds along with behavioral traits that allow avoidance of defenses.

It should be clear that the reciprocal nature of such interactions presents an immediate problem for understanding the evolution of performance at the phenotypic interface of coevolution. Such performance traits

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The Phenotypic Interface of Coevolution

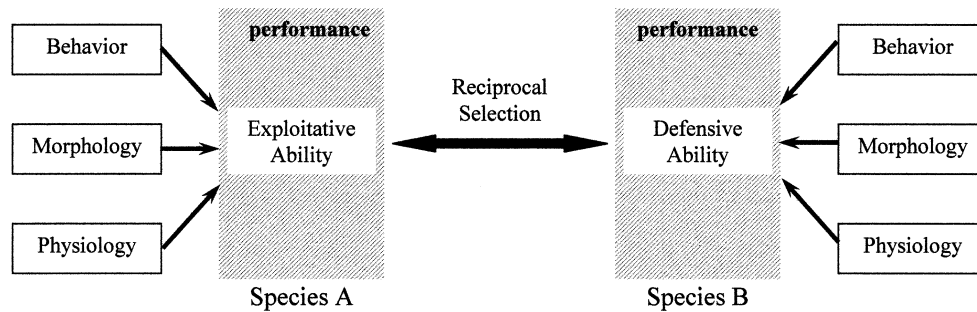


FIG. 1. The phenotypic interface of coevolution. Fitness of each individual involved in interspecific interactions is determined through reciprocal selection both caused and experienced by the phenotypes that mediate the interaction. This phenotypic interface typically involves performance traits that comprise a variety of behavioral, morphological, and physiological components.

do double duty as both targets and agents of selection. In other words, the performance traits at the phenotypic interface simultaneously cause selection on and experience selection by one another. Dissecting the relative importance of the underlying components as targets of selection on the one hand and agents of selection on the other requires simultaneous consideration of complementary performance traits in each of the two interacting species.

Our goal in this paper is to suggest an approach to quantifying and visualizing selection on performance traits at the phenotypic interface of coevolution. This challenge requires understanding how traits in one species influence performance and consequently fitness in an interaction, as well as understanding which traits of the coevolutionary partner influence fitness through interactions with specific traits in the focal species. We suggest that fitness in one species can be expressed as a function of not only the traits present in those individuals, but also as a function of the traits of the coevolutionary partner with which it interacts. The basic technique builds on the covariance approach to understanding selection and allows expression of functions as surfaces so that reciprocal selection can be visualized and more intuitively interpreted.

We first outline the general theoretical approach to the problem and then suggest a method for determining the critical fitness functions from empirical data. The approach is based on direct observation of fitness or performance in interactions between pairs of individuals in both species for which phenotypic values are known. Because we are unaware of the existence of such data sets, we illustrate the approach with hypothetical data based on a well-studied empirical system, an arms race between garter snakes (*Thamnophis sirtalis*) and toxic newts (*Taricha*) (Brodie and Brodie, 1999b; Brodie *et al.*, 2002). Some *a priori* knowledge of the phenotypic interface, such as is available for the newt-snake system, is needed to identify the performance traits of interest and their underlying components. Using this sort of information, expected recip-

rocal selection functions can be deduced and data simulated to illustrate the estimation of these functions.

MODELING SELECTION AT THE PHENOTYPIC INTERFACE OF COEVOLUTION

Theoreticians have devoted considerable energy to modeling coevolutionary interactions (see, *e.g.*, Abrams, 2000; Thompson *et al.*, 2002; Waltman *et al.*, 2002), but these treatments are primarily motivated by questions about evolutionary dynamics, population demography, or stability. A much smaller subset of models has focused on the phenotypic interface of coevolution. These efforts generally have been attempts to describe process and make predictions rather than suggestions for empirical and analytical approaches to understanding the phenotypic interface. Almost no attempts to describe selection on quantitative traits that might be applicable to performance traits have been offered. Kiestler *et al.* (1984) addressed this issue for a plant-pollinator system and Saloniemi (1993) also investigated quantitative trait evolution in a predator-prey model. However, both treatments (Kiestler *et al.*, 1984; Saloniemi, 1993) assumed very specific frameworks prior to the analysis of the model (both models assumed Gaussian stabilizing selection), which preclude the potential to estimate forms and targets of selection.

In a single species context, the covariance approach to estimating selection on multiple quantitative traits has proven invaluable and has revolutionized our understanding of natural selection (Lande and Arnold, 1983; Brodie *et al.*, 1995a; Kingsolver *et al.*, 2001). The two basic problems for understanding selection on multiple traits are that the relationship between individual traits and fitness must be revealed and that trait values are typically correlated within an organism. Lande and Arnold (1983; Lande, 1979) solved these issues by combining the covariance approach of Price (1970, 1972) with the multivariate analysis of Pearson (1903, 1911) to allow the measurement of directional and nonlinear selection through multiple regression.

The Lande-Arnold method treats the organism as a whole or at least a suite of potentially related traits. Simultaneous consideration of multiple traits allows the partitioning of direct selection and indirect selection on correlated traits. Incorporating quadratic variables into the regression measures selection affecting the mean and the (co)variance of a trait(s) within a population as covariances with relative fitness. An added benefit of these gradient measures of selection is that they describe basic features of a selection landscape, allowing us to visualize the relationship between multiple variables and fitness simultaneously to gain a fuller understanding of the details of selection (Phillips and Arnold, 1989; Brodie *et al.*, 1995a).

Here we extend the general covariance approach to modeling and estimating selection to consider cases wherein the fitness of one species depends on the phenotype of a second species with which it interacts. Our goal is to reveal how traits in one species interact with traits in a second species to determine fitness for each interactant. This interaction between performance traits at the phenotypic interface is the key to understanding how the particular traits in one species influence selection on traits in another.

The general model

Existing models for analyzing selection describe the average relationship between fitness and phenotypes. This average is adequate to predict changes in trait means when the selective environment is viewed as a constant, but many agents of selection exhibit variation in the key variables that determine fitness of a focal species. If traits and selective environments interact nonadditively, then fitness (and therefore selection) is context-dependent (Wolf *et al.*, 2003). In such cases, individual fitness cannot be described as a function of the phenotypes alone, but also must consider the specific value of the selective environment that is encountered by that individual. Fitness then becomes a function of both phenotype and selective environment (Brodie and Brodie, 1999b; Wolf *et al.*, 2003).

For interspecific interactions, the description of fitness must consider the consequences of specific combinations of traits of each species at the phenotypic interface. For two interacting species, A and B, let \mathbf{x} be a vector of phenotypes in species A and \mathbf{y} be the vector of phenotypes in species B that potentially influence the fitness of species A (*i.e.*, trait x_i from species A interacts with y_j from species B). The average fitness of a population of species A is then

$$\bar{W}_A = \iint_{-\infty}^{\infty} p(\mathbf{x})p(\mathbf{y})W_A(\mathbf{x}, \mathbf{y}) \, d\mathbf{x} \, d\mathbf{y} \quad (1)$$

where $p(\mathbf{x})$ is a multivariate Gaussian normal distribution for species A defined by

$$p(\mathbf{x}) \equiv \sqrt{(2\pi)^{-m}|\mathbf{P}^{-1}|} \exp\left[-\frac{(\mathbf{x} - \bar{\mathbf{x}})^T\mathbf{P}^{-1}(\mathbf{x} - \bar{\mathbf{x}})}{2}\right],$$

and $p(\mathbf{y})$ is the Gaussian normal distribution of phenotypes in species B. The *interaction fitness function*, $W_A(\mathbf{x}, \mathbf{y})$, describes the fitness dependence of individuals of species A on traits of both species A and species B. $W_A(\mathbf{x}, \mathbf{y})$, $p(\mathbf{x})$, and $p(\mathbf{y})$ are all scalar-valued functions.

Equation 1 is easily related to the theoretical work of Lande and Arnold (1983). Because the integral of $p(\mathbf{y})$ and $W(\mathbf{x}, \mathbf{y})$ is evaluated across the entire \mathbf{y} axis, the resulting function is no longer a function of \mathbf{y} . We know therefore that

$$\int_{-\infty}^{\infty} p(\mathbf{y})W_a(\mathbf{x}, \mathbf{y}) \, d\mathbf{y} = W_A(\mathbf{x}). \quad (2)$$

By substituting equation 2 into equation 1, we get

$$\bar{W}_A = \int_{-\infty}^{\infty} p(\mathbf{x})W_A(\mathbf{x}) \, d\mathbf{x}. \quad (3)$$

Equation 3 is identical to equation 2b from Lande and Arnold (1983), thus all the derived theory for equation 3 (*cf.*, Lande and Arnold, 1983) holds for equation 1 as well. Most notably, an alternate of Price's equation can be found to describe the relationship between selection, relative fitness, and traits at the phenotypic interface of coevolution. Normally, Price's equation states that selection can be quantified as the covariance of relative fitness and phenotype (Price, 1970, 1972). However, because we define fitness in terms of phenotypes of a second species (as in equation 1), the formulation of Price's equation now becomes

$$s_{A|B} = \text{Cov}\left[\mathbf{x}, \frac{1}{\bar{W}_A} \int_{-\infty}^{\infty} p(\mathbf{y})W_A(\mathbf{x}, \mathbf{y}) \, d\mathbf{y}\right]. \quad (4)$$

The integral over all \mathbf{y} can be thought of as the average fitness of an individual with phenotype \mathbf{x} interacting with every individual in a second species, and assumes that individuals interact with individuals from the second species at random. Should individuals interact non-randomly, as, for example, plant-pollinator coevolution where visual or chemical cues exist that mediate the interaction, then $p(\mathbf{y})$ must be redefined to reflect the covariance between interacting individuals (Wolf *et al.*, 2003).

The *interspecific selection differential* for species A, $s_{A|B}$, describes expected directional selection on a vector of traits, \mathbf{x} , considering potential interactions with individuals of species B with a particular distribution of phenotypes, \mathbf{y} . Standard selection measures are based on the specific interactions that each individual experiences, and therefore include a portion of covariance due to interaction effects that are specific to each interaction (Wolf *et al.*, 2003). In comparison, the interspecific selection differential uses that information to describe the expected covariance with fitness for a trait in species A when interacting at random with an entire population of an interacting species B, thereby eliminating sources of covariance between phenotypes

and fitness that result from specific combinations of interacting individuals in the sample. This feature yields a more accurate measure of expected fitness for a trait if interspecific interactions are random, but might be less appropriate if individuals interact assortatively and this covariance is not incorporated *a priori*. Another advantage of this measure is that selection on phenotypes of species A can be investigated when interacting with different distributions of species B phenotypes by integrating over different $p(\mathbf{y})$. Similarly to standard selection analyses, interspecific selection gradients ($\beta_{A_x|B_y}$ and $\gamma_{A_{xx}|B_y}$) can be estimated that reflect selection on specific traits independent of correlated traits.

Estimating the interaction selection function

The key to quantifying selection in an interaction is determining the form of the interaction fitness function, $W_A(\mathbf{x}, \mathbf{y})$, that describes selection on phenotypes of species A due to traits in species B. For each population in which selection is to be measured, three pieces of information must be gathered concerning the phenotypic interface and its consequences: (1) fitness, or some close correlate of fitness that is affected by the interaction, must be observed for each individual in the sample; (2) individual phenotypic values for the focal species must be known; (3) trait values for the individuals in the second species with which each focal individual interacted must be known. The necessity of knowing both trait values in the focal individual and those partners with which it interacts, makes data collection difficult. Researchers *must* observe interspecific interactions among individuals to collect the requisite data. To measure reciprocal selection between species, a fitness correlate due to the interaction in the second species must be obtained as well. The same phenotypic values are used, because traits at the phenotypic interface act as both targets and agents of selection. The traits chosen for the analysis should be those that are considered important to the interaction between the species and any other traits that are potentially highly correlated to those considered important.

The distributions of traits in each species, $p(\mathbf{x})$ and $p(\mathbf{y})$ can be directly observed. Although we assume that these traits are normally distributed, a normal distribution is not critical to the accuracy of predictions made by the model (Lande and Arnold, 1983; Turelli and Barton, 1994). Distributions of \mathbf{x} and \mathbf{y} can be transformed to more closely approximate normality, however, caution must be exercised in the interpretation of the transformed data. The multivariate distribution of the data ($p(\mathbf{x}) p(\mathbf{y})$) determines the space in which interpolation of the interaction fitness function $W(\mathbf{x}, \mathbf{y})$ is possible; outside of this space extrapolation is performed and may be less accurate (sometimes severely). In short, it is important to collect fitness data over as wide of a range of interactions as possible to accurately predict evolution (*cf.*, Wade and Kalisz, 1990).

The relative interaction fitness function, $w(\mathbf{x}, \mathbf{y})$ (*i.e.*, measured as relative fitness rather than absolute fitness), can be estimated in a variety of ways, but the most familiar approaches to evolutionary biologists will be the use of a polynomial least-squares regression (typically quadratic) or a cubic spline (Lande and Arnold, 1983; Schluter, 1988; Brodie *et al.*, 1995a). Regression techniques are relatively easy to implement and provide quantitative estimates of selection that fit models of evolutionary change, but often fail to explain the complexity of real surfaces (Schluter, 1988; Schluter and Nychka, 1994; Brodie *et al.*, 1995b). Cubic splines sometimes provide a substantially better fit to real data than linear regression. Cubic splines are piecewise-smooth families of fourth order polynomials used to create surfaces that can be smoothed to avoid “over-fitting” of data. Because parameter values of the function are not used in the analysis per se, cubic splines are ideal for curve fitting, but do not provide quantitative estimates of selection gradients that are analogous to evolutionary models (Brodie *et al.*, 1995b). Other methods, such as the use of orthogonal functions (*e.g.*, Fourier series, Legendre polynomials), may be used for creation of a surface as well (Hildebrand, 1974).

ENACTING THE PROGRAM IN AN EMPIRICAL SYSTEM

The data requirements for implementing the methodology above are not complicated, but are stringent. Beyond the logistical difficulties of sampling interactions among individuals of two species, one must have some *a priori* knowledge of the phenotypic interface and the traits that comprise it. We are unaware of any currently available data sets that are sufficient for analysis with this model. Instead, we show how information on performance traits at the phenotypic interface can be brought to bear on the methodology by describing the phenotypic interface of an interaction between toxic newts and their garter snake predators. This system is relatively well-studied and provides a complex set of interactions among traits and across species that illustrates many of the features of the model.

Newts of the genus *Taricha* possess large quantities of tetrodotoxin (TTX) in their skin and other tissues (Mosher *et al.*, 1964). TTX is an extremely potent neurotoxin that binds to sodium channels in nerves and muscles and blocks the propagation of action potentials (Hille, 1992; Narahashi, 2001). Toxicity of *Taricha* varies among species and populations, but some individuals possess enough TTX to kill approximately 25,000 white mice or up to 10–15 humans (Brodie *et al.*, 1974). TTX in such concentrations as occurs in *Taricha* is lethal to all known potential predators, with the exception of one species of garter snake, *Thamnophis sirtalis* (Brodie *et al.*, 2002). Resistance to the effects of TTX is the critical phenotype that allows *Thamnophis sirtalis* to engage in a coevolutionary arms race with *Taricha*.

Phylogenetic and geographic patterns of variation in snake resistance to TTX indicate considerable evolu-

tionary lability. Compared to ancestral states, resistance to TTX is somewhat elevated among all natricine snakes examined, but is 100–1,000 times greater in some western populations of *T. sirtalis* (Motychak *et al.*, 1999). Within *T. sirtalis*, extreme resistance to TTX has evolved at least twice, and varies over three orders of magnitude across the species range (Brodie *et al.*, 2002; Janzen *et al.*, 2002). Across the five populations where data on both snake resistance and newt toxicity are available, a close phenotypic match between prey defense and predator resistance is evident, suggesting spatially variable reciprocal selection. Unfortunately, this correlation among phenotypes of predator and prey is the most direct evidence available that a spatial mosaic of selection exists (Brodie *et al.*, 2002). To truly test the existence of hotspots and coldspots of reciprocal selection, and more importantly to evaluate their effects on phenotypic evolution among populations, quantitative estimates of the strength and targets of selection are required.

The phenotypic interface of coevolution between newts and snakes revolves around TTX. On the newt side of the interaction, the amount of TTX in the skin is probably the key trait, but almost nothing is known about the source of TTX or the factors that control its concentration and delivery in newts (Brodie and Brodie, 1999b, c). At present, we treat TTX toxicity as a simple trait, but, in practice, there is probably a complex set of underlying characters that interact to determine toxicity.

On the snake side of the interface, resistance to TTX combines a variety of physiological, behavioral and morphological factors. Organismal resistance to TTX is measured in terms of the effect of TTX on an individual's crawl speed (Brodie and Brodie, 1990; Brodie *et al.*, 2002). This bioassay provides a measure that is precise and repeatable, but more importantly reflects the ecological context of TTX intoxication. Reduced locomotor performance (in fact, complete immobility in extreme cases of intoxication) represents a serious ecological cost to a snake in terms of thermoregulatory ability and escape from predation. Impaired muscle control and coordination is also one of the first organismal level symptoms of TTX poisoning.

The relationship between the amount of TTX ingested (or injected in experimental trials) and the percentage of maximum performance is consistent across populations (Brodie *et al.*, 2002). The best-fit curve is an S-shaped logistic function, and reflects the fact that doses of TTX above or below critical values for a given population do not affect variance in resistance (*i.e.*, above a critical dose, snakes are always immobilized, below a critical dose, snakes are always unaffected). Consequently, these outer limits of the dose range do not represent quantities of TTX that impart selection on resistance phenotypes.

Many factors likely underlie the organismal level of TTX resistance, but two explain much of the variance within and among populations. Among populations, variation in the K_d , or amount of toxin required to

block 50% of sodium channels in skeletal muscle preparations, correlates closely with the organismal TTX resistance. Within populations, variation among individuals in the relative action potential rise rate of skeletal muscles exposed to TTX is linearly related to, and explains 40–50% of the variance in, organismal TTX resistance. These data suggest that differences in skeletal muscle sodium channels are a major contributor to organismal performance, but that other factors also contribute to the observed variation (Geffeney *et al.*, 2002).

Body size also scales linearly with organismal resistance, at least among older juveniles and adults (Ridenhour *et al.*, 2003). The reason for this effect on resistance is unclear, but may be due to a simple dilution effect. In a larger individual the same amount of toxin constitutes a lower concentration, and thus could reduce the binding rate of toxin in tissues. In any case, the positive linear relationship between size and resistance means that a newt of any given toxicity represents a different selective challenge for snakes of different ages or sizes.

Organismal resistance in snakes is accompanied by a tradeoff with crawl speed. At both the individual and family level, speed is negatively associated with resistance such that the fastest snakes are also the least resistant within a population (Brodie and Brodie, 1999a). The proximate cause of the tradeoff is unknown, but might be related to the differences in skeletal muscle physiology that influence resistance. Although this tradeoff probably does not impact the selection due to the interaction with newts, it might represent opposing indirect selection from the snake's own predators, thereby influencing selection on traits comprising the phenotypic interface.

Interactions between newts and snakes are mediated behaviorally as well. After initially seizing and beginning to swallow a newt, many snakes subsequently release the newt after as much as one hour of holding the prey item in the mouth and upper digestive tract (Williams *et al.*, 2003). The longer a snake holds onto a toxic newt the more toxin it is exposed to. Feeding trials with snakes of known resistance have demonstrated that the single predictive factor in acceptance or rejection of a newt and in the amount of time that a snake holds on to the prey is the snake's own resistance. In this manner, snakes may avoid the severe consequences (*e.g.*, death or complete immobilization) of ingesting an overly toxic newt. This assessment of relative prey toxicity may be mediated by a simple physiological response to intoxication, but nonetheless represents another component to the phenotypic interface that determines the strength of reciprocal selection on resistance.

Organismal resistance is a performance trait that is closely related to snake fitness in an interaction with a newt. Given this close correlation, we can construct a hypothetical surface that represents how snake fitness is influenced by interaction with newts of variable toxicity levels.

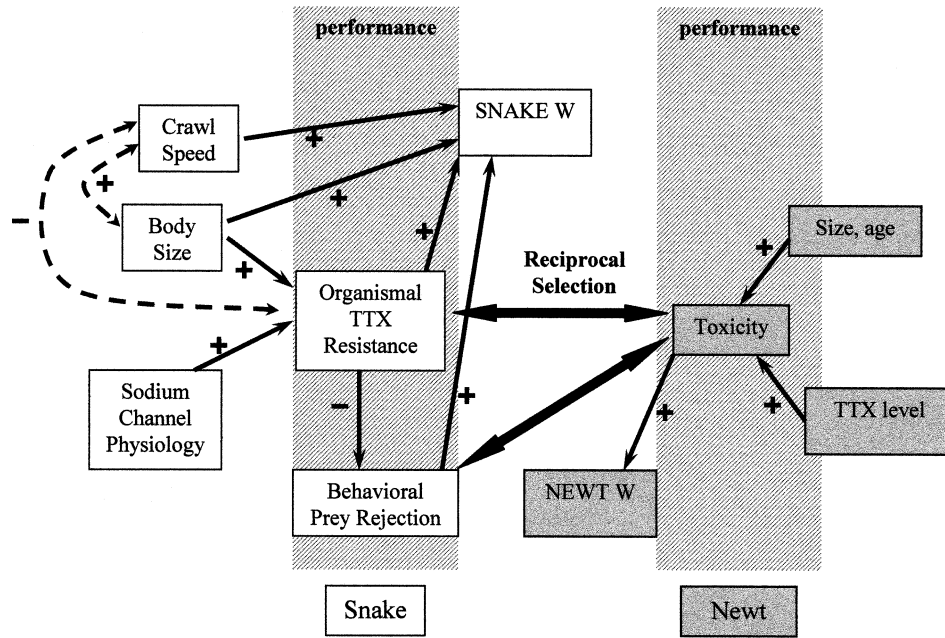


FIG. 2. The phenotypic interface of interactions between garter snakes (*Thamnophis sirtalis*, shown in white) and toxic newts (*Taricha granulose*, shown in gray). The phenotypic interface of coevolution in this predator prey interaction centers on the level of tetrodotoxin (TTX) in the newt and organismal resistance in the snake. However, several components influence organismal resistance in snakes. Organismal resistance itself negatively influences a second dimension of the phenotypic interface, the tendency to behaviorally reject prey that are too toxic.

Analyzing the interaction selection function

By combining the relationships learned through previous studies (Fig. 2) we developed a hypothetical interaction fitness function $W(\mathbf{x}, \mathbf{y})$ for garter snakes. Using this function, we generated a simulated data set to illustrate the methodology and interpretation of the parameters. Because the data set is simulated, some features of real data (such as deviations from multivariate normality, and stochastic sampling effects that introduce covariances) are minimized. The function incorporates the two major factors influencing organismal level resistance (skeletal muscle resistance [sm] and mass), the toxicity of prey interacted with, and a trade-off between performance and a trait under positive selection outside the interaction (speed):

$$W = \left\{ \frac{1}{1 + \exp \left[1.01 \text{Log} \left(\frac{67998 \text{toxicity}}{\text{mass}} + 1 \right) - \text{sm} - 3.61 \right]} - 15 \text{toxicity} \right\}^3 + 0.15 \text{speed} + \varepsilon \quad (5)$$

Each of the four traits (skeletal muscle resistance, mass, speed, and toxicity) used in the analysis had a unique normal distribution standardized to means of zero and unit variance from which values were picked. Two hundred pairs of data points were sampled from $p(x)$ and $p(y)$ (each pair therefore represents an observed interaction). Using the phenotypes \mathbf{x} and \mathbf{y} , fit-

ness was then calculated for the interaction based on equation 5. A normally distributed error ($\sim N(0,0.01)$) was then added to W to represent error attributable to other fitness benefits and measurement error.

We begin by estimating the shape of the expected interaction fitness surface $\hat{w}(x, y)$ as a function of both \mathbf{x} and \mathbf{y} traits. We generated our expected function using cubic splines because they generally provide better fit to more complex surfaces. Our surface is five-dimensional and cannot be visualized for all traits simultaneously, but a series of three-dimensional plots captures the character of the surface (Figs. 3, 4). It is possible to use multivariate techniques to reduce the number of dimensions of the surface (Phillips and Arnold, 1989; Schluter and Nychka, 1994) but such approaches eliminate some aspects of interactions between individual traits and often are difficult to interpret in the context of the original phenotypic interface. Because we fit a simulated set of data, it is unsurprising that we should recover a function very similar to the hypothesized $W(\mathbf{x}, \mathbf{y})$. The interpretation of these surfaces will be discussed below.

To quantitatively estimate selection in the interaction, we conduct a standard quadratic regression analysis (Lande and Arnold, 1983; Brodie *et al.*, 1995b) including the phenotypic interface traits of both species. This analysis yields selection gradients on \mathbf{x} in species A and interaction gradients for \mathbf{y} in species B. Because the regression includes traits not part of the focal species, the gradients cannot be interpreted equivalently to standard Lande-Arnold gradients. For example, an apparent directional selection gradient for

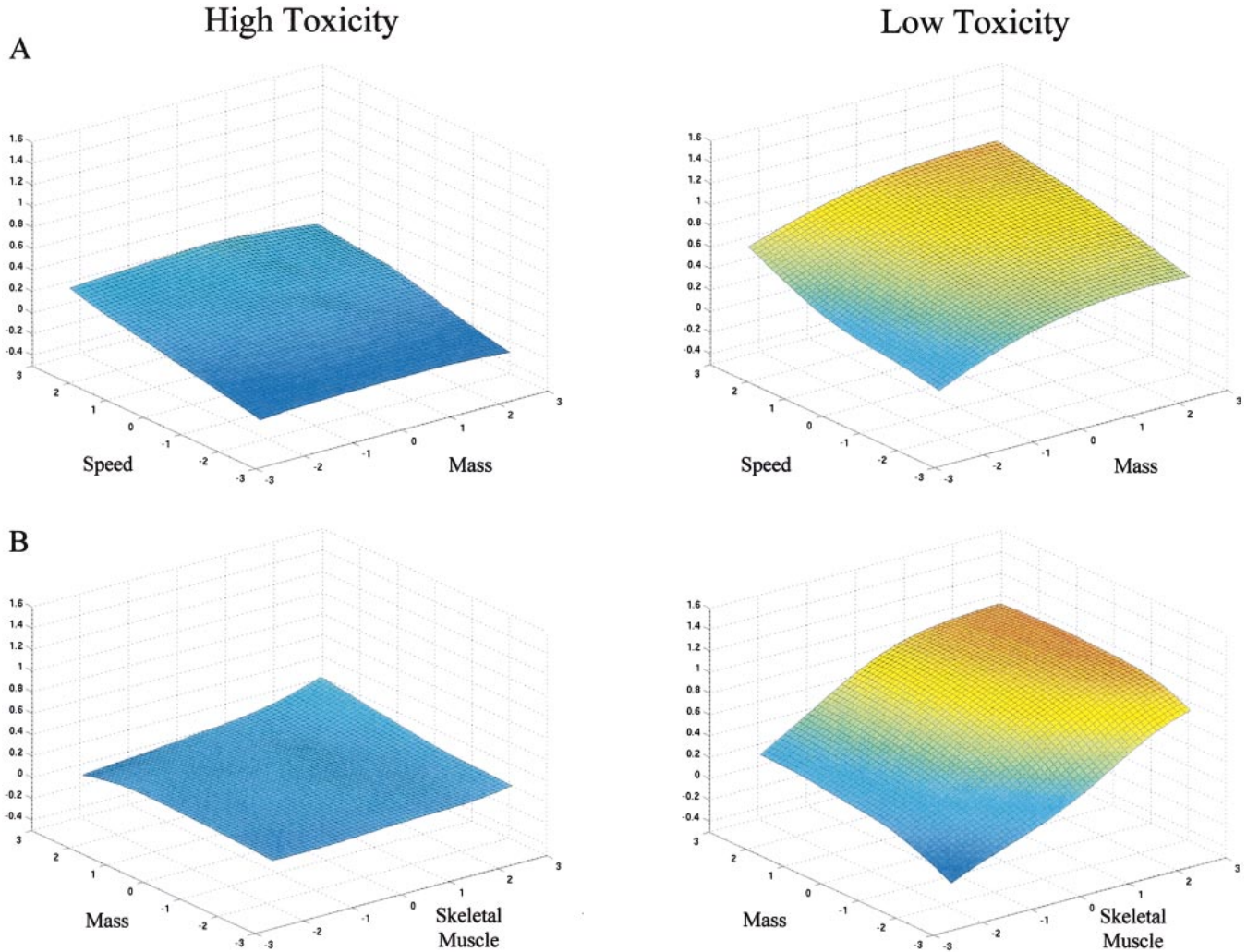


FIG. 3. Fitness surfaces for snake phenotypes in two average contexts. Surfaces showing the relationship between snake fitness and snake phenotypes are shown for populations interacting with high toxicity prey (left) and low toxicity prey (right). Identical colors indicate same fitness levels in all plots. In the first series (A), speed and mass both positively influence fitness, and the shape of the surface does not differ across general context. In the second series (B), mass and skeletal mass resistance both positively influence fitness. Average context of the interaction alters the overall shape of the surface because expected fitness is uniformly low in the high toxicity case, preventing interactions between traits from having substantial effects on fitness.

trait y , β_y , does not imply selection in the usual sense. In this case, the measured covariance (the “interaction gradient”) is between fitness in species A and phenotype, y , in species B, so the gradient describes incremental effects on fitness in species A given a change in the phenotype with which an individual interacts. As long as no covariance exists between traits in species A and the traits with which they interact in species B (*i.e.*, all interactions occur at random), the regression coefficients relating fitness and phenotype within a species yield the standard selection gradients, β and γ . Together, the interaction selection gradients and interaction gradients describe the interaction fitness surface particular to the observed set of interactions between individuals.

To understand relationships with fitness in the context of the whole population of the interacting species,

we estimate the interspecific selection function and related parameters, beginning by removing dependence upon y from the fitness data by averaging $w(x, y)$ over y (equation 4). Thus, we calculate the expected relative fitness, \hat{w} , for a given x_i using the equation

$$\hat{w}(x_i, \cdot) = \frac{1}{n} \sum_{j=1}^n \hat{w}(x_i, y_j).$$

Each *interspecific fitness function*, $\hat{w}(x_i, \cdot)$ represents the expected relative fitness of x_i averaged over all the possible interactions with y . In the context of our example, $\hat{w}(x_i, \cdot)$ measures the average relative fitness expected for a snake with a value i for trait x , when it interacts with all possible newts in a population with the phenotypic distribution $p(y)$. For empirical data, we would use the $p(y)$ measured in the interacting population. However, it is also possible here to substitute

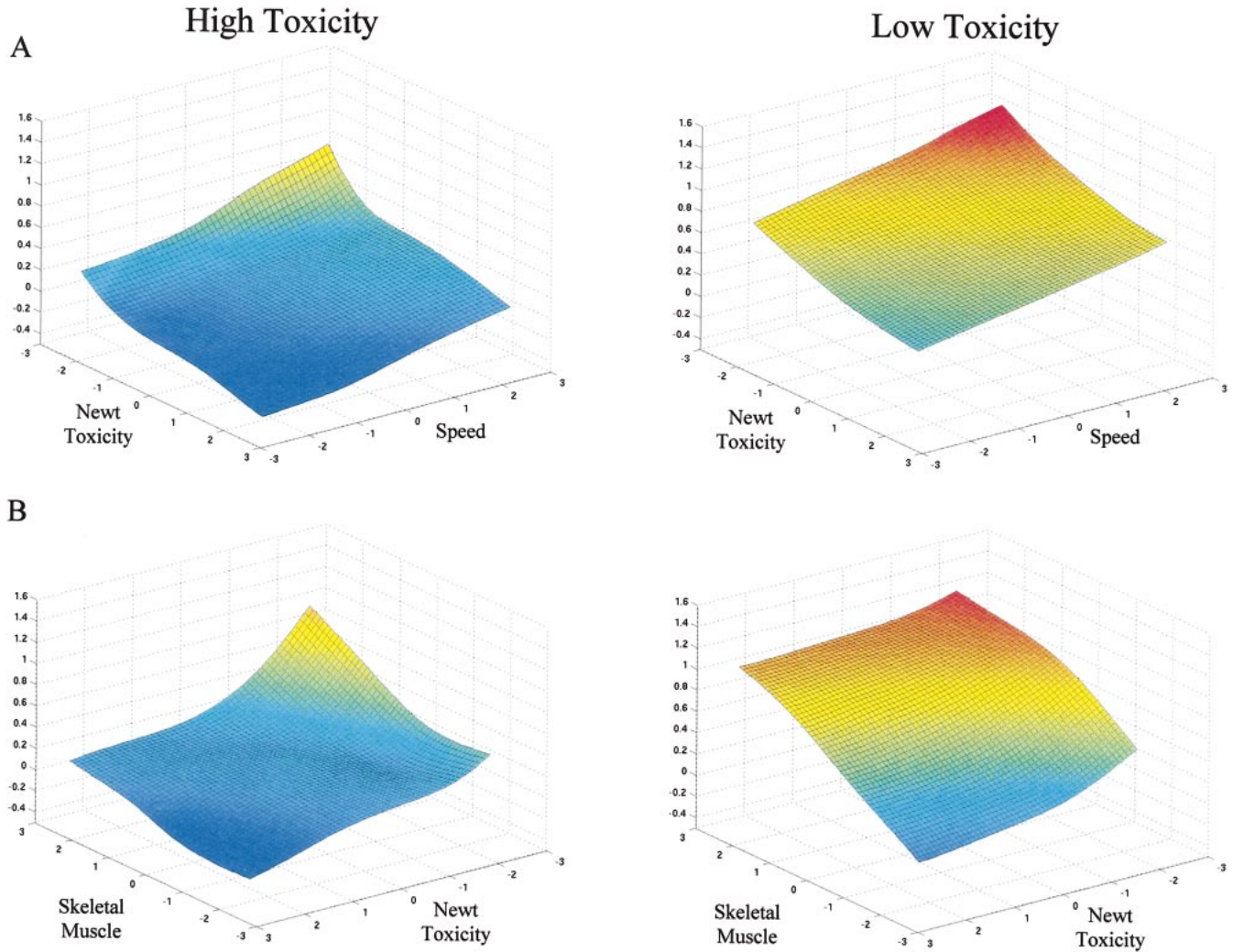


FIG. 4. Interaction fitness surfaces for combinations of snake and newt traits in two average contexts. Surfaces relate snake fitness to both snake phenotypes and newt phenotypes assuming populations of newts with high toxicity (left) and low toxicity (right). Identical colors indicate same fitness levels in all plots. In the first series (A), speed is shown to positively influence fitness in both cases. Both surfaces are relatively flat showing little (high toxicity) or no (low toxicity) bivariate curvature in either average context, indicating that speed and toxicity of newts do not interact in a non-additive fashion to influence snake fitness. In the second series (B), skeletal muscle resistance positively affects snake fitness in both cases, but the shape of the surface differs markedly between high and low toxicity newts. Against high toxicity newts, skeletal muscle resistance and toxicity interact non-additively as evidenced by the severe bivariate curvature to the surface. Against low toxicity newts, this non-additive interaction disappears, leaving a flatter fitness surface.

other distributions to determine how selection would change if, for example, snakes interacted with newts of higher or lower resistance (see below).

The expected interspecific fitness $\hat{w}(x, \cdot)$ is next regressed on x to yield the *interspecific selection gradients*, $\beta_{A|B}$ and $\gamma_{A|B}$. The linear and nonlinear *interspecific selection differentials*, $s_{A|B}$ and $C_{A|B}$ can be estimated from the analogous covariances. These parameters reflect the expected selection on traits in one species when individuals interact at random with individuals in a second species. These estimates differ from the parameters from the standard Lande and Arnold type regression in that they eliminate covariances with fitness due to specific combinations of traits of individual interactants.

Interpreting the surface and parameters

The interaction fitness function $W(\mathbf{x}, \mathbf{y})$ allows for interpretation of the phenotypic interface between interacting species. Interspecific interactions make selection context-dependent at two levels. Within populations of interacting species the individual context of pairwise interactions influences selection. Among populations, differences in the average context can influence the general mode of selection for the population as a whole. Therefore the major goals of such an analysis are to determine which traits in a partner species interact with specific traits in the focal species, and how changes in context both within and between populations can change the form and strength of selection on all traits. Both visual inspection of the interaction

TABLE 1. *Linear selection gradients and differentials.*

		High toxicity		Low toxicity	
		β	s	β	s
Interaction selection coefficients	Speed	0.532	0.431	0.091	-0.022
	Mass	0.090	0.068	0.088	0.100
	Skeletal muscle	0.273	0.061	0.263	0.232
	Newt toxicity	-0.408	-0.384	-0.127	-0.125
		$\beta_{A B}$	$S_{A B}$	$\beta_{A B}$	$S_{A B}$
Interspecific selection coefficients	Speed	0.530	0.403	0.097	-0.014
	Mass	0.077	0.103	0.092	0.110
	Skeletal muscle	0.280	0.066	0.267	0.232

fitness surface and quantitative estimates of the selection and interaction gradients can be used to evaluate selection in an interspecific interaction. Context-dependence is revealed in both cases by signatures of non-additive interactions between traits in the focal species and traits in the partner species:

(1) *Visual comparisons.* Non-additive effects will be apparent in terms of curvature in more than one dimension. As with standard selection surfaces, curvature indicates nonlinear fitness effects due to a combination of variables. When one axis is the selective context (e.g., prey toxicity), nonlinearity indicates that selection on a focal trait is dependent on the phenotype of the individual partner in the interaction.

(2) *Selection coefficients.* Selection and interaction gradients provide quantitative estimates of features of selection described above. Just as nonlinear selection gradients, γ_{xx} , provide estimates of correlational selection, the bivariate non-linear interaction gradients, γ_{xy} , provide a measure of the dependence of selection on the interaction context. Interaction selection differentials provide similar information that does not consider the effects of correlations among traits. Linear and univariate terms are interpreted as in standard selection analysis (Lande and Arnold, 1983; Brodie *et al.*, 1995a).

(3) *Comparisons of selection and interspecific selection coefficients.* Standard selection coefficients are sensitive to the effects of specific interactions among individuals, whereas interspecific selection gradients and differentials, estimated from $\hat{w}(x_i, \cdot)$, describe fitness relationships after removing the effect of context. A comparison of analogous selection and interspecific selection coefficients provides a measure of the importance of context within a set of interactions. If context has a strong effect on any particular form or target of selection, the analogous interspecific coefficient should differ in magnitude and/or sign.

(4) *Comparisons of average population context.* By evaluating selection with respect to different distributions of traits for the interacting species, the importance of the average context is revealed. For example, if we evaluate selection on the performance trait TTX resistance in garter snakes, selection might differ between populations interacting with high toxicity prey *versus* low toxicity prey. Such comparisons can be

achieved experimentally by manipulating biological interactions, or hypothetically by evaluating equations with respect to different interactant distributions. Differences in analogous surfaces, gradients, and differentials between average contexts will illustrate how selection changes as a function of the population with which the interaction occurs.

To illustrate the signatures of context-dependence, and the dramatic effects that it might have on selection at the phenotypic interface, we examine selection based on our hypothetical interaction fitness function for garter snakes and newts. As suggested above, we estimate selection in two different average contexts—a population of high toxicity newts where means of newt toxicity and snake resistance are closely matched, and a population of low toxicity newts where the mean of newt toxicity is lower than the mean snake resistance.

We first examine how average prey context influences selection on combinations of snake characters alone. Speed influences fitness outside of resistance, while mass influences fitness through resistance to TTX (Fig. 2). With respect to high toxicity newts, we see that speed experiences positive directional selection and does not interact with mass to affect fitness (Fig. 3A). Selection on speed is much weaker when interacting with low toxicity newts. Quantitative estimates of directional selection gradients support this interpretation and differ between the average contexts examined (Table 1, compare β_{speed}). Selection gradients and interspecific selection gradients are virtually identical, indicating no context dependence (Tables 1, 2 compare β_{speed} *vs.* $\beta_{\text{speed|B}}$). These results are consistent with a trait that influences fitness outside of the phenotypic interface and therefore is not expected to experience context-dependent selection. An effect of average context is observed, probably due to the importance of the phenotypic interface to fitness when interacting with low toxicity newts. Note that the general shape of the selection surfaces is the same in both contexts, only the slope of the surface with respect to speed changes.

Body mass and skeletal muscle resistance both influence snake fitness through whole animal resistance. Both traits experience positive directional selection against either high or low toxicity newts, with selec-

TABLE 2. *Nonlinear interaction selection gradients (γ_{xy}) and interspecific selection gradients ($\gamma_{A/B}$).*

		High toxicity				Low toxicity			
		Speed	Mass	Skeletal muscle	Newt toxicity	Speed	Mass	Skeletal muscle	Newt toxicity
Interaction selection gradients	Speed	-0.045	0.021	-0.031	-0.019	0.000	-0.004	0.001	0.008
	Mass		-0.018	0.072	-0.064		-0.050	-0.006	0.021
	Skeletal muscle			0.034	-0.103			-0.078	0.033
	Newt toxicity				0.246				0.038
		Speed	Mass	Skeletal muscle		Speed	Mass	Skeletal muscle	
Interspecific selection gradients	Speed	-0.009	0.006	-0.013		-0.001	-0.006	0.001	
	Mass		-0.013	0.058			-0.049	-0.010	
	Skeletal muscle			-0.026				-0.077	

tion much stronger on skeletal muscle resistance (Fig. 3B). Nonlinear selection favors positive combinations of skeletal muscle resistance and mass in interactions against high toxicity newts (Table 2). This form of selection all but disappears in interactions with low toxicity newts, leaving primarily linear selection in that average context (Fig. 3B). A comparison of interaction and interspecific selection coefficients further supports this relationship, as the bivariate nonlinear interaction selection gradient is smaller than the analogous gradients in high toxicity populations (Table 2). Against high toxicity newts, linear selection differentials are less than analogous interspecific selection differentials (Table 1), again indicating that the context of individual interactions is important in determining the strength of selection on mass and skeletal muscle resistance. These results are consistent with selection that is dependent on the toxicity of newts that individual snakes interact with, and shows that the context can alter the shape of the multivariate selection surface.

Incorporating traits of another species provides a direct analysis of the relationship between fitness in one species and phenotype of another, as it relates to the phenotypic interface. Again, we consider speed as a character whose fitness effects are not expected to be mediated through an interaction with toxicity of prey. Once again we see that the general shape of the interaction fitness surface is similar regardless of toxicity of the prey, though directional selection is minimal when interacting with low toxicity newts (Fig. 4A). Nonlinear gradients indicating context dependent fitness related to prey toxicity are nearly zero (Table 2, $\gamma_{\text{speed, toxicity}}$). Quantitative estimates of nonlinear gradients are nearly identical in both average contexts. Interspecific selection gradients involving speed are equivalent to the interaction selection gradients, further indicating no dependence of selection on speed upon the toxicity of newts.

Note that interaction gradient and differential estimates are obtained for toxicity (Table 1). These estimates can be interpreted statistically similarly to selection gradients. Fitness of the focal species is related to variation in toxicity, but these should not be considered estimates of selection, because fitness and phe-

notype are not present in the same population. Our analysis shows that fitness of snakes is negatively linearly (β_y) related to the toxicity of individual newts that they interact with in both contexts. Some (concave) curvature in this relationship is also apparent in the estimates of the γ_{yy} terms (Table 2).

The surfaces and terms that examine the relationship between toxicity, skeletal muscle resistance and snake fitness illustrate the pattern expected for traits directly involved in the phenotypic interface for each species (Fig. 4B, Tables 1, 2). With respect to high toxicity newts, the interaction fitness surface reveals curvature in the direction of opposing combinations of toxicity and resistance ($\gamma_{\text{skeletal muscle, toxicity}} < 0$). This effect is not only reduced, but to a small degree reversed (curvature in the direction of positive combinations, $\gamma_{\text{skeletal muscle, toxicity}} > 0$) when snakes are compared to low toxicity newts. Thus, fitness within one set of interactions is clearly dependent on the context of the interactant's phenotype, but the form of this effect depends strongly on the average context of interaction. When snakes are much more resistant than newts are toxic, the degree of context dependence is reduced because most snakes can successfully interact with most newts.

CONCLUSION

Selection that results from interactions between species is the engine of coevolution. Traits that evolve in coevolutionary interactions do so because they constitute a phenotypic interface that mediates the outcome of individual interactions. Such traits are typically complex performance characters that involve a network of underlying components. Understanding the evolution of performance is one key to understanding the phenotypic interface of coevolution.

Because interactions allow for context dependence, one of the special problems of understanding coevolutionary selection is revealing this context. Which traits interact to affect fitness in a focal species, and how changes in context can alter selection on traits are the first order questions in dissecting coevolutionary interactions. We suggest a visual and statistical approach that can be adopted to address these questions. Although we envision the problem in terms of biotic

interactions and contexts that are the phenotypes of other species, similar context dependence might apply in any situation where selection on performance traits depends on a variable environment. Context might also involve interactions among more than two species, as in the case of mimicry rings or guilds of herbivores or pollinators. The approaches outlined herein could easily be extended to examine such situations.

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