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Research article

Measuring natural selection on proportional traits: comparisons of three types of selection estimates for resistance and susceptibility to herbivore damage

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Abstract. To compare the strength of natural selection on different traits and in different species, evolutionary biologists typically estimate selection differentials and gradients in standardized units. Measuring selection differentials and gradients in standard deviation units or mean-standardized units facilitates such comparisons by converting estimates with potentially varied units to a common scale. In this note, I compare the performance of variance- and mean-standardized selection differentials and gradients for a unique and biologically important class of traits: proportional traits, that can only vary between zero and one, and their complements (1 minus the trait) using simple algebra and analysis of data from a field-study using morning glories. There is a systematic, mathematical relationship between unstandardized and variance-standardized selection gradients for proportional traits and their complements, but such a general relationship is lacking for mean-standardized gradients, potentially leading investigators to mistakenly conclude that a proportional change in a trait would have little effect on fitness. Despite this potential limitation, mean-standardized selection differentials and gradients represent a useful tool for studying natural selection on proportional traits, because by definition they measure how proportional changes in the mean of a trait lead to proportional changes in relative fitness.

Key words: fitness elasticity, natural selection, proportional traits, resistance, selection gradients, standardized selection gradients

Introduction

One of the major accomplishments of evolutionary biology over the last 30 years has been the development of methods to readily estimate natural selection, visualize it, and evaluate the accuracy of those estimates (Lande, 1979; Lande and Arnold, 1983; Arnold and Wade, 1984a, b; Schluter, 1988; Phillips and Arnold, 1989; Simms, 1990; Wade and Kalisz, 1990; Rausher, 1992; Schluter and Nychka, 1994; Brodie *et al.*, 1995; Janzen and Stern, 1998; Scheiner *et al.*, 2000; Stinchcombe *et al.*, 2002). The most common approach to estimating natural selection is to regress an estimate of relative fitness (absolute fitness divided by the mean fitness of the population) onto the traits of interest. The resulting partial regression coefficients are termed selection gradients (usually symbolized as β), and provide an estimate of the relationship between the trait and fitness, controlling for correlations between the focal trait and other traits included in the regression model (Lande and Arnold, 1983; Rausher, 1992).

Comparisons between selection gradients for different traits are difficult because the units of unadjusted selection gradients – the units of the trait to the -1 power – differ in a way that can obscure comparisons. To choose an example from plants, an estimate of selection on plant height might be in units of cm^{-1} or m^{-1}, while an estimate of selection on branch number would be in units of branches^{-1}. Given this difference in units, it is difficult to determine whether an unstandardized selection gradient of 0.5 branches^{-1} is of greater or lesser magnitude than an unstandardized selection gradient of 0.4 cm^{-1}. To ease comparisons across traits and species, and alleviate any confusion introduced by the different units of selection gradients for different traits, selection gradients are often presented in standardized, dimensionless units (see, e.g., Endler, 1986; Kingsolver *et al.*, 2001; Geber and Griffen, 2003 for three reviews making such comparisons). By standardizing the data, it is possible to estimate the relative strength of selection on different traits, each with different natural units, in the same selection model.

Two approaches can be used to estimate standardized selection gradients – standardizing by the standard deviations of the traits or standardizing by the means of the traits. The first approach, which is more common, simply involves dividing the traits by their standard deviations prior to use in the regression with relative fitness, or equivalently, multiplying the unstandardized selection gradient by the standard deviation of the trait, i.e., $\beta' = \beta\sigma$ (e.g., Lande and Arnold, 1983). In this formulation, the effect of the trait on relative fitness is put in standard deviation units. That is, for a standard deviation change in the trait, how much change is there in relative fitness? Another approach, which has been recently proposed (Morgan and Schoen, 1997; van Tienderen, 2000; Hansen *et al.*, 2003; Hereford *et al.*, 2004), involves dividing the traits by their means prior to analysis, or equivalently, multiplying the unstandardized selection gradient by the mean of the trait, i.e., $\beta_e = \beta\bar{z}$, where $\bar{z}$ is the mean of the trait. In this formulation, selection gradients are expressed as fitness elasticities (thus the symbol, β_e). These fitness elasticities indicate the percent change in relative fitness for a proportional change in the mean of the trait. For example, a fitness elasticity of 0.5 would indicate that a 10% increase in the mean of the trait would lead to a 5% increase in relative fitness (Morgan and Schoen, 1997).

In this note, I compare unstandardized, variance-standardized, and mean-standardized selection gradients for a unique and biologically important class of traits: traits that are best estimated as proportions, and as such, only vary between 0 and 1.

Proportions as biologically important traits

Many important biological traits are best estimated as proportions, and examples can be found in a variety of species and areas of biology. For example, for phytophagous insects that use alternate host plants, the fitness of females can be influenced by the relative proportion of host plants upon which eggs are deposited (e.g., Rausher, 1978; Brown *et al.*, 1995; Feder and Filchak, 1999; Filchak *et al.*, 2000; Via *et al.*, 2000). For some protandrous flowering plants (plants that flower first as males, and then as females), natural selection favors individuals that spend a greater proportion of their flowering time in the female phase (Campbell *et al.*, 1994; Campbell, 1996). In addition, plant resistance to herbivores or pathogens is commonly estimated as the complement of percent of leaf area damaged or infected (i.e., 1− percent damage; Rausher and Simms, 1989; Simms and Rausher, 1989, 1993; Stinchcombe and Rausher, 2001, 2002). For some forms of herbivore damage that are qualitative (e.g., apical meristem damage is present versus absent), damage can only be scored as a zero or 1. In these cases, investigators often average these values for multiple individuals within a quantitative genetic family (e.g., Tiffin and Rausher, 1999; Weinig *et al.*, 2003), and then analyze resistance as a proportional trait. Comparing the strength of selection on all of these characters to other traits thus requires estimating selection gradients or differentials for proportional traits.

Methods

To compare the performance of unstandardized, variance-standardized, and mean-standardized selection gradients, I use two approaches: simple algebra and an analysis of field data taken from an experimental study of morning glories.

Algebra

The nature of proportional data immediately suggests one challenge that any estimate of selection should meet: there should be a systematic relationship between selection on the proportional trait of interest and selection on the complement of that trait. In other words, if selection is acting to decrease susceptibility to herbivore damage, a reasonable deduction is that selection is acting to increase resistance to herbivore damage and that the selection gradients for resistance and susceptibility are systematically related to each other. Here I demonstrate that there is a systematic, mathematical relationship between traditional unstandardized and standardized selection gradients for proportional traits and their complements, but that such a systematic mathematical relationship is lacking for fitness elasticities.

The general formula for a regression coefficient is:

$$\beta_1 = \frac{\mathrm{COV}(w, z)}{\mathrm{VAR}(z)} \tag{1}$$

in which w is relativized fitness and z is the trait of interest. Substituting the complement of a proportional trait (i.e., resistance for susceptibility) and expanding Equation (1) yields:

$$\beta_2 = \frac{\sum(w_\mathrm{i} - \overline{w})((1 - z_\mathrm{i}) - (1 - \overline{z}))}{\sum((1 - z_\mathrm{i}) - (1 - \overline{z}))^2} \tag{2}$$

The denominator of Equation (2) simplifies to: $\sum$ $[-1(z_\mathrm{i} - \overline{z})]^2$, which is equivalent to the denominator of Equation (1). However, simplification of the numerator yields: $-1\sum (w_\mathrm{i} - \overline{w})(z_\mathrm{i} - \overline{z})$. In other words, if selection on susceptibility to pathogen damage equals β, selection on resistance to pathogen damage equals $-\beta$.

Similar algebra demonstrates that the standard deviation of susceptibility and resistance will be equal, i.e., $\sigma_\mathrm{z} = \sigma_{(1-\mathrm{z})}$, and as such the standardized selection gradient for resistance, $\beta_{(1-\mathrm{z})}'$, is equal to the standardized selection gradient for susceptibility, β_z', multiplied by -1. One benefit to these relationships is that the magnitude of selection on various traits can be assessed by examining the absolute value of the selection gradients without the need to explicitly consider the sign of the gradient (e.g., Kingsolver *et al.*, 2001).

The relationship between fitness elasticities for resistance and susceptibility, however, will rarely be so well behaved. For example, the fitness elasticity for susceptibility will equal $\beta\overline{z}$, but the fitness elasticity for resistance will equal $-\beta\ (1 - \overline{z})$. Thus, if the fitness elasticity for susceptibility is $\beta\overline{z}$, the fitness elasticity for resistance will be $-\beta + \beta\overline{z}$. These results lead to two potential problems. First, there will only be a systematic relationship between the fitness elasticity for susceptibility and the fitness elasticity for resistance in cases where $\beta = 0$ or $\overline{z} = 0.5$, and this represents a potential limitation to the use of fitness elasticities. Second, the lack of a consistent relationship between the elasticities can obscure interpretation. For example, if selection is acting in favor of resistance (i.e., $\beta_{1-\mathrm{z}}$, $\beta_{1-\mathrm{z}}'$, and $\beta\ (1 - \overline{z}) > 0$), calculating a fitness elasticity for susceptibility could be biased closer to zero by adding a negative number ($-\beta$, when $\beta > 0$). Accordingly, one could conclude that a proportional change in susceptibility would have little effect on relative fitness. In contrast, if one analyzed the fitness elasticity of resistance, one would conclude that proportional increases in resistance would lead to increases in relative fitness.

Field methods

To illustrate how the results presented above are likely to be manifested in empirical situations, I estimated unstandardized, variance-standardized, and mean-standardized selection differentials and gradients from a field experiment with the Ivyleaf morning glory (*Ipomoea hederacea*). Full field methods are described by Stinchcombe (2002), and here I analyze previously unpublished data from the control treatment of that experiment. Briefly, the control treatment of that experiment comprised 266 plants, grown from seed in the field in a randomized, blocked design. Seeds planted in the experiment were derived by letting 20 inbred lines self-fertilize, thereby mimicking the natural mating system of *I. hederacea*. Plants were separated from each other by 1 m, and were given a 2-m wooden garden stake to twine around.

The focal traits I analyze here are a resistance trait (resistance to mature-leaf insect herbivore damage) and a plant size trait (estimated total leaf area), both estimated 7 weeks after seedling emergence. Resistance to mature-leaf insect herbivore damage was estimated as 1 minus the percent of leaf area damaged by herbivores (1 − % damage), while total leaf area was estimated by multiplying the average leaf area of four leaves (approximately 20% of the leaves on a typical plant; Stinchcombe, 2002) by the total number of leaves for each plant. Fitness was estimated as the number of viable seeds set per plant, and was relativized by dividing by the mean seed set of the control treatment. In all selection models, I use inbred line means to reduce the influence of environmental covariances between traits and fitness (e.g., Rausher, 1992; Stinchcombe *et al.*, 2002). To minimize the influence of spatial variation on the estimates of line means, inbred line means were calculated as least-square means from statistical models that included the effects of block.

I estimated selection differentials from regressions of relative fitness on the trait of interest with no other terms in the model, and present unstandardized (s), variance-standardized (i), and mean-standardized selection differentials (s_e). Summary statistics for the traits analyzed are presented in Table 1. To evaluate the strength and direction of natural selection after accounting for selection on other traits, I estimated selection gradients from multiple regression models that included either leaf area and resistance, or leaf area and susceptibility as the independent variables. Resistance and leaf area exhibited a positive, but non-significant inbred line-mean correlation ($r = 0.26$, $p = 0.27$). Because preliminary analyses indicated tentative evidence that the residuals from these multiple regression models were significantly different

Table 1. Summary statistics for the variables analyzed, calculated from the inbred line means ($N = 20$ inbred lines)

Trait (units)	Mean	SD	Min	Max
Susceptibility (% leaf area damaged)	0.036	0.014	0.016	0.058
Resistance (1 − % leaf area damaged)	0.964	0.014	0.942	0.984
Leaf area (cm^2)	678.05	193.20	308.48	969.01

from normal ($A^2 = 0.699$, $p = 0.0593$ by Anderson-Darling test), I present selection gradient estimates from models with relative fitness as the response variable, but test the statistical significance of these estimates from models using $\log(y + 1)$ transformed relative fitness (Mitchell-Olds and Shaw, 1987).

Results and discussion

Analysis of the field data from morning glories suggests that natural selection is acting to increase leaf area and resistance to herbivore damage (or to decrease susceptibility), although selection on resistance (susceptibility) is not significant at the $p < 0.05$ level in either analysis (Tables 2 and 3). Comparison of the selection differentials and selection gradients suggests that accounting for natural selection on leaf area reduces the estimate of natural selection on resistance (susceptibility); in other words, a portion of the total selection on resistance, estimated by the selection differentials, is due to indirect selection on leaf area. In general, the selection differentials and gradients reveal similar patterns about the pattern of natural selection as estimated by the three types of selection estimates.

At first glance, the unstandardized gradients appear to suggest that selection is acting much more strongly on resistance/susceptibility than on leaf area. However, because of the disparate units for these selection estimates, such a direct comparison is difficult. The standardized gradients, which have comparable units, suggest that natural selection is acting more strongly on leaf area – a standard deviation change in leaf area would have the effect of increasing relative fitness by approximately 0.21, while a similar standard deviation change in resistance would only increase relative fitness by approximately 0.09.

Table 2. Selection differentials for resistance, susceptibility, and leaf area, estimated with unstandardized data (s), variance-standardized data (i), and mean-standardized data (s_e)

Trait	s (s.e.)	i (s.e.)	s_e (s.e.)	p-value
Resistance	9.99 (5.18)	0.14 (0.07)	9.62 (4.99)	0.07
Susceptibility	−9.99 (5.18)	−0.14 (0.07)	−0.36 (0.18)	0.07
Leaf area	0.0012 (0.0003)	0.23 (0.06)	0.81 (0.21)	0.0013

Table 3. Selection gradients for resistance, susceptibility, and leaf area, estimated with unstandardized data (β), variance-standardized data (β'), and mean-standardized data (β_e)

Trait	β (s.e.)	β' (s.e.)	β_e (s.e.)	p-value
(A)				
Resistance	6.23 (4.25)	0.0889 (0.06)	6.01 (4.1)	0.23
Leaf area	0.00107 (0.003)	0.2074 (0.06)	0.728 (0.21)	0.0042
(B)				
Susceptibility	−6.23 (4.25)	−0.0889 (0.06)	−0.226 (0.15)	0.23
Leaf area	0.00107 (0.003)	0.2074 (0.06)	0.728 (0.21)	0.0042

(A) Selection gradients from a model including only resistance and leaf area. (B) Selection gradients from a model including only susceptibility and leaf area. *P*-values were estimated from models with relative fitness that had been $\log(y + 1)$ transformed.

The mean standardized selection gradients also measure the strength of natural selection on different traits in comparable units, and these selection gradients suggest that proportional changes in resistance have much more dramatic effects on relative fitness than do proportional changes in leaf area. For instance, according to these data, a 1% change in the mean level of resistance would have the effect of increasing relative fitness by 6.01%, while a similar proportional change in mean leaf area would only have the effect of increasing relative fitness by 0.7%. In large part, the discrepancy between the variance-standardized and mean-standardized estimates of selection are due to the unique nature of proportional traits. Because proportional traits are bounded between 0 and 1, their standard deviations are also mathematically bounded between 0 and 1. Accordingly, the variance-standardized estimate of selection on a proportional trait will always be reduced compared to the unstandardized estimate. Because the mean of proportional traits is also bounded between 0 and 1, mean-standardized selection gradients will also be reduced when compared to unstandardized selection gradients. Whenever the standard deviation of a trait is less than the mean, the variance-standardized estimates will also be reduced compared to mean-standardized selection gradients.

The potential complications of estimating mean-standardized gradients for proportional traits are also illustrated by Tables 2 and 3. While the unstandardized and variance-standardized selection gradients and differentials for resistance and susceptibility equal each other multiplied by −1, there is no such simple relationship between the fitness elasticities. For instance, the fitness elasticities for resistance in the multiple regression model (Table 3) suggest that a 1% change in resistance would lead to ≈6% increase in relative fitness. However, the fitness elasticity for susceptibility suggests that a 1% increase in susceptibility would lead to ≈0.23% decrease in relative fitness. Fortunately, in this case the fitness elasticity of resistance/susceptibility to mature-leaf insect damage is not statistically significant at the $p < 0.05$ level, so it is likely that any interpretations about the relative strength of selection on resistance/susceptibility *versus* leaf area would be tentative anyway. However, there is no clear *a priori* reason why similar problems will not arise in other cases in which a resistance or susceptibility elasticity is statistically significant.

Although it may appear that changing one's focus from resistance to susceptibility merely reorders the axis upon which the trait is measured, such reordering can have dramatic impacts on interpretations about fitness consequences of changes in the mean value of a trait. While in this case switching from considering susceptibility instead of resistance to herbivore damage might have led to an underestimate of the strength of selection, this will not always be the case. As described earlier, the fitness elasticity for susceptibility will equal $\beta \bar{z}$, but the fitness elasticity for resistance will equal $-\beta\,(1-\bar{z})$. Thus when the mean level of damage is greater than 0.5, the absolute value of the fitness elasticity for susceptibility will always be greater than the absolute value of the fitness elasticity for resistance. However, when the mean level of damage is less than 0.5, the situation will be exactly reversed: the absolute value of the fitness elasticity for resistance will always be greater than the absolute value of the fitness elasticity for susceptibility. Given these potential difficulties, fitness elasticities for proportional traits should be used with caution that they are not biased towards the conclusion that proportional changes in the traits will have little or no effect on relative fitness.

Despite the absence of a simple, general relationship between fitness elasticities for resistance and susceptibility, they remain a useful tool for studying natural selection on proportional traits, provided that investigators ensure that their results are not biased closer to zero. Because fitness elasticities, by definition, measure how proportional changes in the mean of a trait lead to proportional changes in relative fitness, they are naturally suited for traits that are best measured as proportions. Moreover, because proportional traits will typically have small standard deviations relative to their means, fitness elasticities can potentially reveal that a trait is more important for relative fitness than would be expected based on variance-standardized selection estimates. For example, the field data from morning glories suggests that increases of a single standard deviation of leaf area would have appreciably greater effects on relative fitness than a single standard deviation change in resistance. In contrast, the fitness elasticities reveal that a 1% change in resistance would have dramatically greater effects on relative fitness than a 1% change leaf area. Comparison of the variance-standardized selection estimates and fitness elasticities reveals that resistance to herbivore damage is not unimportant for the fitness of plants, but rather that there is dramatically less variation in resistance to herbivore damage than leaf area.

Given the potential limitations of both deviation- and mean-standardized selection estimates, what then is the practicing evolutionary ecologist to do?

Estimating the strength of natural selection on proportional traits and comparing it to other types of traits is likely to remain an important empirical and statistical challenge that will not go away. For some cases in which the traits have dramatically different means, variances and distributions, comparisons will be difficult on any standardization scale – for example, consider the possible complications of comparing the strength of selection on continuous, proportional, and multinomially distributed traits. Hereford *et al.* (2004) argue that mean-standardized measures are likely to be superior in these instances, given the dramatic differences in the variances of these distributions. However, in addition to the problems discussed above, proportional traits pose an additional challenge: for many proportions estimated from binomial outcomes, there will be a correlation between the mean and variance. The mean-variance correlation inherent to many proportional traits undercuts one of the main motivations for developing mean-standardized selection gradients, which was to standardize estimates of β by terms that were independent of those used to estimate β in the first place (i.e., for $\beta' = \beta\sigma$, σ is obviously not independent of the trait variance used in estimating β; Hereford *et al.*, 2004). As Hereford *et al.* (2004) point out, it is unlikely that any single standardization scheme will serve all purposes.

However, several possible solutions exist. First, if the goal of an investigation is to quantitatively compare the strength of natural selection on proportional and non-proportional traits, investigators should report all of the necessary data for readers to come to their own conclusions: the unstandardized, variance-standardized, and mean-standardized selection estimates. In this manner readers and reviewers can come to their own conclusions about whether a given standardization approach is unduly affecting the conclusions of a study (Hereford *et al.*, 2004). Second, if investigators use the mean-standardized approach for proportional traits, it should be done in a manner that prevents an underestimation of the fitness elasticity of the trait. Third, investigators should be cautious in concluding that a low variance-standardized selection gradient indicates that a trait is unimportant for fitness. Because standardized selection gradients measure the impact of selection relative to the variability in a trait, it is possible that there is simply not much variation in the trait in the population under study (e.g., the comparison between the variance- and mean-standardized selection gradients for leaf area and resistance).

As many authors have noted (e.g., Houle, 1992; Kirkpatrick, 1996; Morgan and Schoen, 1997; van Tienderen, 2000; Hansen *et al.*, 2003; Hereford *et al.*, 2004), the standard equations of evolutionary genetics can often be transformed in a manner that refocuses our attention on different aspects of evolutionary change, whether change is measured in the original units of the trait, standard deviations of the traits, or relative to the original mean of the trait. While these tools allow comparisons across species, traits, and classes of traits (e.g., behavioral versus morphological), it is important to ensure that the scale of measurement does not unduly affect our interpretations.

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