

Quantifying nonadditive selection caused by indirect ecological effects: Reply

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We appreciate the opportunity to reply to Bolstad's (2017) comment on our paper, "Quantifying nonadditive selection caused by indirect ecological effects" (terHorst et al. 2015). We respectfully disagree with Bolstad's argument that our method does not properly quantify nonadditive selection in response to indirect ecological effects, as it certainly does for many of the biological scenarios we envision. However, as with all tests related to ecological indirect effects, the appropriateness of our approach depends on the underlying model that best describes the multispecies interaction. Specifically, the null model we propose applies only to fitness effects of multispecies interactions that are best modeled with additive rather than multiplicative models. The In-transformations Bolstad proposes are appropriate for constructing null models of nonadditive selection when the underlying species interactions are best modeled multiplicatively. The use of additive vs. multiplicative models has a long history in the study of indirect ecological effects (Case and Bender 1981, Billick and Case 1994, Wootton 1994b), which we revisit briefly here and extend to measures of selection. Specifically, we clarify our goals and approach and address Bolstad's two major criticisms of our paper: (1) that we incorrectly infer indirect ecological effects from our measure of the non-additivity of selection, and (2) that log fitness (in contrast to fitness relativized across treatments as we propose) is more appropriate for quantifying selection in response to multiple species over varying timescales. We explain our reasoning and use a simple model to demonstrate when our null model is appropriate and when Bolstad's log fitness model is most appropriate for identifying nonadditive selection in multispecies communities.

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First, to clarify the goals of our original paper, we provided a method to detect and estimate the strength of nonadditive selection, which occurs when selection on a trait imposed by one selective agent is altered in the presence of a second selective agent. Our goal was *not* to use evidence for nonadditive selection to infer indirect ecological effects; ecologists have many simpler methods available for detecting indirect ecological effects (Strauss 1991, Wootton 1994a, Menge 1995) that do not require the large sample sizes and additional trait measurements needed to understand natural selection. Most studies estimate the consequences of indirect ecological effects for species abundances, rather than fitness or selection (Miller and terHorst 2012), and rarely measure traits. Indirect ecological effects do not necessarily result in non-additivity of selection, and nonadditive selection can result from processes other than indirect ecological effects. For example, an indirect ecological effect that alters fitness will not necessarily also result in nonadditivity of selection on the traits under consideration. Rather, for any species to alter selection on another it must alter the covariance between relative fitness and a trait and, not just affect mean fitness (Wade and Kalisz 1990, Strauss et al. 2005).

Conversely, nonadditive selection can arise via two mechanisms: (1) indirect ecological effects, the mechanism of interest to most ecologists, and (2) reductions in mean fitness in the presence of multiple interacting species that are accompanied by reduced variance in fitness and, therefore, reduced opportunity for selection on traits. For example, in cases where antagonists dramatically reduce fitness, there may be little or no opportunity for selection because all individuals have zero (or nearly zero) fitness, providing no variation in fitness upon which selection can act. This mechanism may have been at least part of the cause of the non-additivity of selection on resistance in the first case study in our paper. In short, although indirect ecological effects can result in nonadditivity of selection, the presence of nonadditive selection is not evidence for indirect ecological effects, nor is the presence of indirect effects evidence for nonadditive selection.

Second, Bolstad argues that fitness should be relativized within populations or natural log-transformed because the effects of multiple ecological interactions are best modeled as multiplicative effects. The relative merits of multiplicative vs. additive models in estimating the strength of indirect ecological effects have been thoroughly discussed and debated, and depend on the biotic interaction under investigation and the fitness components measured (Case and Bender 1981, Billick and Case 1994, Wootton 1994b). Below, we describe scenarios where additive models are likely most appropriate, where multiplicative models are likely most appropriate, and

cases where both models provide accurate tests for non-additive selection. We then extend our simple model to illustrate these predictions.

Indirect ecological effects can be generally classified into interaction chains and interaction modifications (Wootton 1993). Interaction chains, often called density-mediated indirect effects (Abrams 1995), result when a species alters the outcome of interactions between two other species simply because it alters the abundance of one of those species. In such chains, species only directly affect the fitness of adjacent species in the chain, while indirectly affecting the fitness of non-adjacent species. Interaction modifications, often called trait-mediated indirect effects (Abrams 1995), result when a species alters *how* two other species interact, without affecting their abundances. These types of indirect effects are not mutually exclusive, but in either case, these indirect ecological effects will cause non-additive selection if the interactions influence the

relationship between relative fitness and the traits that mediate the strength or likelihood of the interaction.

For many interaction chains, both additive and multiplicative models will be appropriate because one of the species will not exert selection on the focal species (Fig. 1a). For example, in trophic cascades (Fig. 1a), apparent competition, and even indirect mutualisms, if species A does not directly influence selection on species C, but indirectly affects species B (which does exert selection on C), then there is only one selective agent and therefore no fitness effects to multiply, but nonadditive selection can still occur. Here, an additive model or multiplicative null model will yield the same results because only species B is a selective agent on species C. In these cases, an additive model may be preferred given the problems with using ln-transformed fitness in selection analyses noted previously (Stanton and Thiede 2005).

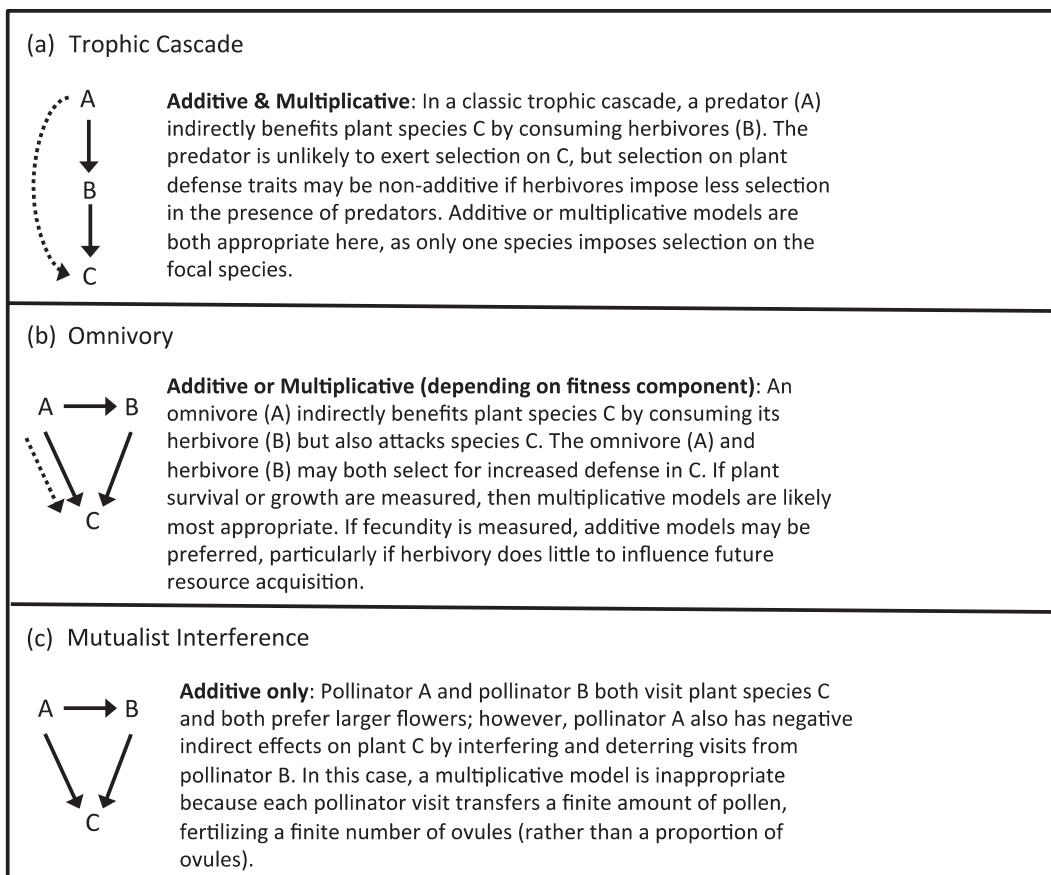


FIG. 1. The choice of whether to use multiplicative or additive models for testing the effects of species A and B on selection on species C depends on the biology of the system, including whether both A and B influence species C's fitness and are selective agents on species C, the fitness component measured, and the timing of the interactions. Arrows depict effects on mean fitness, solid for direct and dashed for indirect effects. Species will impose selection only if the studied traits mediate the likelihood or outcome of the interaction. In some cases, non-additive selection can result even in the absence of indirect effects on mean fitness if a species alters the trait-fitness relationship. Panel (a) illustrates one case where both additive and multiplicative models are appropriate because species A is unlikely to exert selection on C. Panel (b) illustrates a case where the appropriate model depends on the fitness component measured. Panel (c) illustrates the case where an additive model is most appropriate.

Similarly, in some types of interaction modifications both additive and multiplicative models may apply. In a case where a plant species provides herbivores refuge from predators, the plant species may not influence herbivore survival or selection on herbivore traits directly but will reduce the effects of predation on survival and likely the strength of predation as a selective agent. As previously noted, here only the predator exerts selection on the herbivore, and although the presence of plants may influence the strength of predation and the strength of predation as a selective agent, there are no fitness effects to multiply.

In other scenarios (both interaction chains and modifications; Fig. 1b), both species may exert selection on the focal species, and in this case whether an additive or multiplicative model is more appropriate depends on the fitness component measured. Some fitness components, like survival, are typically combined multiplicatively. For example, if species A reduces species C survival by 70% and species B arrives and reduces species C survival by 50%, species C survival probability would be predicted to be 0.15 ($0.3 \times 0.5 = 0.15$) rather than the impossible value of -0.2 ($1 - 0.7 - 0.5 = -0.2$). Similarly, growth over time is often exponential, so differences in growth rate should also typically be modeled multiplicatively.

Some ecologists would argue that because fecundity is so closely linked to growth, fecundity should also be modeled multiplicatively; however, we can think of numerous cases where multiplicative models seem a poor fit for the actual biology, especially in single generation selection studies where population growth of interacting species is unlikely to come into play (Bender et al. 1984). For example, because pollinators carry a finite amount of pollen, increased visitation by pollinators will increase the number of ovules pollinated (Fig. 1c). Each additional pollinator will transfer some additional number of pollen grains. In this case, a multiplicative model based on proportion of ovules pollinated and seeds set makes little biological sense, although in cases where plants are rarely pollen limited and where each additional pollinator visit results in a smaller number of ovules fertilized, multiplicative models may be appropriate (Bolstad 2017). Similarly, in cases of seed predation, a given density of seed predators is likely to remove a set number (rather than proportion) of seeds. Even in cases where biomass or resources are removed via parasitism or herbivory, an additive model may make sense if biomass removal occurs at a time when additional resources are being transferred to fruit production rather than growth. Indeed, this is the basis of the model we presented in the Appendix of our original paper. Here we revisit our initial model and then present a modified model that uses survival as the fitness component to illustrate when log-transformed fitness (multiplicative models) vs. non-transformed fitness (additive models) are best used in tests of nonadditive selection.

Our model simulates the null hypothesis of purely additive selection, that is, two selective agents whose effects are entirely independent. In our original simulation, two herbivore species consume the same plant species and are deterred by the same plant defensive trait (trichome number). We created a plant population consisting of 99 plants that all have equal sized leaves with a normal distribution of trichome numbers (mean = 50, standard deviation = 10). We modeled attack by herbivores on each plant by assuming that trichomes influence the amount of leaf material consumed by each herbivore species in a deterministic fashion, such that the percent leaf damage from Herbivore 1 is $100 - \text{trichome number}$ and the percent leaf damage from herbivore species 2 is $100 - (0.5 \times \text{trichome number})$. That is, trichome defenses are 50% less effective in reducing attack from Herbivore 2 relative to Herbivore 1. Note that damage by each herbivore is independent of damage by the other herbivore.

In the absence of herbivores, plants produce a maximum of 1,000 seeds. We include costs of trichome production in the absence of herbivores by reducing fitness by two seeds for each trichome produced. Leaf damage by each of the two herbivores has equal and independent fitness effects—each percent of leaf consumption from each herbivore reduces seed production by five seeds regardless of the level of damage by the other herbivore. Thus, there are no ecological indirect effects either through consumption or the fitness effects of that consumption. Note that in this scenario, as in many of the systems we reviewed in our original paper, the effects of each species on individual fitness are additive rather than multiplicative. We present results for multiplicative effects of selective agents on fitness below. We estimated linear variance standardized selection differentials by regressing relative fitness (each individual fitness value divided by mean fitness) on standardized trichome number (Lande and Arnold 1983). Previous work has relativized fitness either across all treatments, dividing by the grand mean fitness, or within each treatment, dividing by mean fitness within each treatment. Here we evaluated both methods, as well as Bolstad's method of using ln-fitness.

When fitness was relativized across treatments, the simulation revealed weak selection against trichome number when both herbivores were absent, due to the cost of producing trichomes (Table 1). There was strong selection for increased trichome number when only Herbivore 1 was present, and weaker selection for increased trichome number when only Herbivore 2 was present (Table 1), because trichomes were more effective in reducing damage from Herbivore 1 than Herbivore 2. Recall that Herbivore 1 did not influence the amount of damage from Herbivore 2 (and vice versa), that trichome numbers directly influence damage which influences fitness, and there was no interaction between the effects

TABLE 1. Selection differentials on trichome number in response to different biotic environments.

Selection Differential	Relativized fitness across treatments	Relativized fitness within treatments	ln-Fitness
β_0	-0.036	-0.024	-0.024
β_1	+0.054	+0.049	+0.049
β_2	+0.009	+0.010	+0.010
Additive prediction ($\beta_1 + \beta_2 - \beta_0$)	+0.099	+0.083	+0.083
β_{12}	+0.099	+0.214	+0.230
Deviation from additive	0	0.131	0.147

Notes: Subscripts denote the presence of each herbivore. All analyses were performed on the same simulated data. Selection differentials were estimated from the simulation model that incorporated the effects of two herbivores with independent effects on plant fitness, which should produce the predicted additive effect when both species are present; only relativizing fitness across treatments produces the desired additive effect. The last column represents a multiplicative model, using ln-fitness.

of the two herbivores on fitness in our simulation. As a result, there were no indirect ecological effects, and the selection by each herbivore was not affected by the presence of the other herbivore. Thus, selection should be additive. The simulation indicated very strong selection for increased trichome number when both herbivores were present, with the estimate matching the predicted additive effect (Table 1).

Bolstad's method of using ln-fitness does not recover the null hypothesis of independence of selection imposed by the two herbivores, as simulated (Table 1). Under Bolstad's method, selection when both herbivores were present was consistently stronger than the predicted additive selection, suggesting that this method will lead to erroneously high estimates of nonadditive selection, and Type I errors when testing the significance of the nonadditive selection gradient. Bolstad suggests that the method we proposed is almost guaranteed to find non-additive selection in response to indirect ecological effects. If the species interactions studied are additive in nature, this is certainly untrue and our model suggests that erroneously detecting nonadditive selection is more likely using ln-fitness. Further evidence that our method does not inevitably lead to nonadditive selection comes from the empirical examples in our original paper. Our two case studies find additivity for one of the two traits in each case, and only two of four previous studies we cited reported evidence for nonadditive selection.

In other cases, the fitness consequences of multiple ecological interactions may best be modeled multiplicatively. We modified our above simulation to simulate such a scenario. In this case, we model plant cuticle thickness as a trait that influences the probability of infection from two pathogens. Each pathogen reduced

the likelihood of survival by 50%, and increasing cuticle thickness reduces the likelihood of infection, although the trait was 20% less effective in reducing the likelihood of infection by pathogen B. We modeled the joint fitness effects of infection by both pathogens with a multiplicative model. For example, the probability of a plant surviving if infected by both pathogens is 25% (0.5×0.5), instead of 0, as in an additive model. As previously noted, the pathogens do not interact with each other, so no indirect ecological effects occur and all other model assumptions are the same (we included a cost of cuticle thickness, cuticle thickness is normally distributed, etc.). The model is deterministic, so rather than using logistic regression to estimate selection gradients, the model is analogous to a genotypic selection analysis regressing family survival probabilities in each treatment on family mean cuticle thickness. In this case, the selection differential calculated using relative fitness across all treatments when both species are present fails to recover the additive prediction (Table 2). However, when fitness is ln-transformed, as suggested by Bolstad, the selection differential matches the null hypothesis. Assuming appropriate underlying models of species interactions is key to employing our null model in tests for non-additive selection arising from ecological indirect effects.

There is no simple answer as to whether an additive or multiplicative model is more appropriate for testing for nonadditive selection in biological communities. This decision depends on the fitness component under consideration, the timing of the interactions, and whether there are multiple agents of selection. Even in cases where we argue that an additive model is most appropriate (Fig. 1c), there are likely exceptions, based on the nature of the biological interactions (e.g., the pollen-ovule

TABLE 2. Selection differentials on cuticle thickness in response to different biotic environments.

Selection Differential	Relativized fitness across treatments	Relativized fitness within treatments	ln-Fitness
β_0	-0.015	-0.011	-0.011
β_1	0.060	0.060	0.060
β_2	0.046	0.048	0.049
Additive prediction ($\beta_1 + \beta_2 - \beta_0$)	0.121	0.119	0.120
β_{12}	0.084	0.109	0.120
Deviation from additive	-0.037	-0.010	0

Notes: Subscripts denote the presence of each herbivore. All analyses were performed on the same simulated data. Selection differentials were estimated from the simulation model that incorporated the effects of two pathogens with independent effects on plant fitness, which should produce the predicted additive effect when both species are present; only using a multiplicative model and ln-fitness produces the desired additive effect.

example described above). However, when selection is very weak, both additive and multiplicative models produce similar null models.

We appreciate Bolstad's interest in our paper and his comment that illuminated when relative fitness vs. In-transformed fitness should be employed when using our approach to test for non-additive selection. Ultimately, the choice of additive vs. multiplicative models will be system specific and depend on the nature of the interacting species and the components of fitness that are measured. We hope that our clarification of the methods will be helpful to empiricists planning to implement our approach in future studies. Our suspicion is that many readers of *Ecology* already have data on hand, or could easily modify their experimental design or the data they collect, to quantify nonadditive selection. More estimates of the strength of nonadditive selection in natural communities are needed to determine the relative importance of pairwise, additive, and nonadditive selection in driving evolution in a community context.

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