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DEFENSIVE SYNERGISMS? REPLY TO PENNINGS

Mark E. Hay[1]

A *synergism* is defined as, "the joint action of different substances in producing an effect greater than the sum of the effects of all substances acting separately" (Funk and Wagnalls 1968). Pennings (1996) argues that (1) the additive null hypothesis used by Hay et al. (1994) to assess synergisms can be impossibly stringent and (2) that this hypothesis has as a critical (and possibly false) assumption that "changes in food quality lead to proportional changes in feeding." He then suggests an alternative method for detecting synergisms (the multiplicative null hypothesis). Although both of Pennings' points have merit, his second point is more important than his first, the problems raised by his second point will often nullify the utility of his suggested alternative methodology, and none of the limitations he discusses affect the particular synergisms documented by Hay et al. (1994).

Pennings contends that the additive null hypothesis used by Hay et al. (1994) is too conservative because it cannot detect synergisms when both defenses are individually very active. The statistical approach used by Hay et al. involved two separate steps. The first was an ANOVA (or equivalent nonparametric procedure) to determine if the deterrent effect of the two defenses in combination was really greater than the effects of either defense alone. In most cases, the deterrent effect of a single defense was as great as the effect of both defenses together, thus indicating that no further analyses were necessary or appropriate because neither synergistic nor additive effects were occurring. As an example, *Rhipocephalus* metabolites alone or $CaCO_3$

[1] University of North Carolina at Chapel Hill, Institute of Marine Sciences, 3431 Arendell Street, Morehead City, North Carolina 28557 USA.

alone reduced urchin feeding by ≈70%, but both together reduced feeding by 88% (see the bottom graph in Fig. 2 of Hay et al. 1994). Before testing to see if this greater apparent deterrency was additive or synergistic, we had to first determine whether the combined deterrency was really any greater than the effects of chemical or mineral defenses alone. This first step in our analyses demonstrated that none of these effects differed from each other ($P = 0.630$). In cases such as this, Pennings (1996) suggests that we will be testing our 88% decrease in feeding against an unobtainable expectation of 140% when, in fact, the first step of the analysis indicates that no test for either synergistic or additive effects is appropriate. In contrast to this, if one applies Pennings' (1996) multiplicative null hypothesis to these same data, the predicted effect needed to define a synergism is 91%, or only 3% more than what we observed. This suggests that Pennings' multiplicative method might detect "synergisms" where differences are neither significant nor additive, much less synergistic.

Mathematics and statistical procedures aside, if an organism has two traits, each of which very strongly depress feeding (the extreme being a 100% depression), then there will be minimal advantage of, or selection for, any synergistic effect. In extreme cases, where a single trait reduces feeding by 100%, synergisms are, by definition, impossible. In all but three of the cases investigated by Hay et al. (1994), the combined defensive value of both $CaCO_3$ and secondary metabolites was not significantly greater than the defensive value of one or the other of these traits in isolation. In these cases, one need not use either the additive or multiplicative null hypothesis to search for synergisms—the initial ANOVA procedure indicates that none are present. The majority of the potentially problematic cases discussed by Pennings were avoided by this initial procedure.

Pennings' second point is important. He notes perceptively that the additive null hypothesis used by Hay et al. (1994) mandates "the critical assumption that multiple changes in food quality lead to proportional total changes in feeding" (Pennings 1996). He con-

tends that this assumption is inappropriate because changes in food quality could cause nonlinear changes in feeding. As examples, as deterrent traits are added to a food, they could be below-threshold levels of detection and thus cause no change in feeding, or they could cross detection thresholds and cause large, nonlinear, decreases in feeding. This is a critical point that will complicate the study of synergisms. Because our study was a first effort to investigate synergisms between potential chemical and mineral defenses, we chose the simple and conservative approach of using the additive null hypothesis (this approach is also consistent with the strict definition of a synergism). Although this approach has limitations, we judged it to be more appropriate than the multiplicative method suggested by Pennings because that method results in all simple additive effects being classified as synergistic. Future studies designed to evaluate possible synergisms might do this most directly and elegantly by using multiple-factor ANOVAs (or modifications of similar tests) in which the interaction term is used to assess synergisms. Billick and Case (1994) provide a thoughtful discussion of statistical procedures that are appropriate for detecting higher order interactions in ecological experiments. Although their focus is on interactions among different species, their procedures and suggestions for avoiding statistical errors also apply to evaluations of potential synergisms. Unfortunately, the design of the multiple paired assay procedures used by Hay et al. (1994) did not allow these types of analyses, so an alternative and more cumbersome methodology had to be devised.

The investigation of potential synergisms is in its infancy and in most cases there are insufficient data to determine what conditions might call for an additive null hypothesis, a multiplicative null hypothesis, or an alternative approach. We can, however, see how using different approaches might have affected interpretation of the synergisms noted by Hay et al. (1994). Pennings cautions that, ". . . the 'synergisms' observed by Hay et al. (1994) could result from consumers disproportionately avoiding less palatable foods rather than from one defense making the other more potent." Although this concern is very reasonable when two deterrent effects are combined and therefore might be pushing the total deterrency across some threshold of detection, this caution is invalid when applied to the particular data generated by Hay et al. (1994). In the cases of synergistic feeding deterrency documented by Hay et al., Pennings' criticism is in error because both cases involved increases in deterrency with the addition of a plant trait that was not at all deterrent in isolation. In one case, two traits that had no effect on feeding when tested alone, became strongly deterrent when they were combined. In the other case, a deterrent trait became

significantly more deterrent when combined with a trait that had no effect on feeding when tested alone. Pennings' caution, however, should be considered in future investigations because many will involve the interactions of several deterrent traits.

The multiplicative null hypothesis that Pennings suggests using involves the assumption that adding multiple negative traits has *less than simple additive effects* on feeding. A justification is not given for this assumption, and it is in direct conflict with his second point where he argues that adding negative traits can have nonlinear effects on feeding and can thus produce deterrent effects that look synergistic, but may not be. Additionally, this approach will identify all simple additive effects as synergistic (see Pennings' [1996] example of a 44% decrease in feeding being defined as a synergism when the simple additive effects alone would be expected to be 50%). This procedure can be misleading and can leave us looking for complex underlying mechanisms where none occur. Future investigations of synergisms should use statistical procedures similar to those discussed by Billick and Case (1994) because those are more useful, rigorous, and elegant than either those used by Hay et al. (1994) or those proposed by Pennings (1996).

However, a meaningful understanding of synergisms will not be achieved by improvements in statistical procedures alone. Synergisms will not be adequately understood until we have an enhanced appreciation for the physiological effects of prey defenses on consumers and more knowledge of the mechanisms involved in creating these effects. Very few marine studies have addressed the direct physiological effects of prey defense on consumers (Boettcher and Targett 1993, Lindquist and Hay 1995), much less the complex, indirect, additive, or synergistic effects. Studies focused on these aspects of consumer–prey interactions would be especially useful.

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