The Overcrowded Stage and the Evolutionary Play:

Resistance of *Brassica rapa* L. (Brassicaceae) to Multiple Enemies

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Abstract of the Dissertation

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Ecosystems contain complex networks of biotic interactions. In order to understand the ecology of a species and the evolution of many of its traits it is important to incorporate many of these interactions into our studies. Plants, for example, are attacked by a plethora of enemies, including vertebrate and invertebrate herbivores, and fungal, bacterial, and viral pathogens. Although we have accumulated knowledge about plant defense mechanisms to particular enemies, we are still far from understanding how plants cope with multiple enemies. Do plants evolve defenses in a specific manner to a given enemy, or do the effects of multiple enemies condition evolutionary responses? I contributed to this field of inquiry with artificial selection experiments using rapid cycling *Brassica rapa* L. (Brassicaceae). I selected populations of *B. rapa* for greater resistance to a fungal pathogen, the cabbage leaf spot, *Alternaria brassicicola*. Lines that evolved greater resistance to *A. brassicicola* did not exhibit correlated resistance to other enemies, particularly to larvae of three lepidopterans (*Pieris rapae*, *Trichoplusia ni*, and *Spodoptera exigua*), adults of a flea beetle (*Phyllotreta cruciferae*), or to the cabbage aphid (*Brevicoryne brassicae*). This suggests the independence of resistance to fungal pathogens and insect herbivores. In addition, I selected lines of *B. rapa* for divergent expression levels of anthocyanin pigments. These play important roles in response to abiotic factors, such as protection from UV light, but also in biotic interactions, for instance providing color to flowers that attract pollinators. I found that lines expressing higher levels of anthocyanins were more susceptible to *P.rapae* and *P. cruciferae* and less susceptible to *T. ni* and *A. brassicicola*. Feeding by *S. exigua* and colony size of *B. brassicae* did not differ among lines producing extreme anthocyanin contents. This presents a varied suite of resistance effects of anthocyanins, and illustrates the complexities of conflicting selection pressures that affect the evolution of plant defense.

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Introduction

Organisms face a number of selection pressures, including selection upon form, physiology and behavior, reproductive performance, and response to abiotic and biotic stresses. An important component of Evolutionary Ecology deals with how organisms adapt to multiple, at times conflicting, selection pressures. For instance, Life History Theory attempts to predict the optimal strategy given different constraints upon life-history parameters, such as age at first reproduction or reproductive rate.

This approach has also been applied to plants with regard to their allocation of resources to different functions, including growth, reproduction and defense. Different patterns of allocation to these competing needs constitute different strategies. Within each of these basic functions, there are multiple components that also compete for resources. Thus one might find trade-offs between aboveground versus belowground growth, or allocation trade-offs to male versus female reproductive structures.

Plants may also allocate resources to different forms of defense. Some plant defense theories have considered how plants might allocate to different chemical defense pools. The Carbon/Nutrient Balance hypothesis (Bryant et al. 1983) models how plans allocate surplus resources to carbon- or nitrogen-based defenses depending on environmental conditions. The Resource Availability (Coley et al. 1985) and the Plant Apparency hypothesis (Feeny 1976; Rhoades and Cates 1976) consider how plants evolved the production of ‘quantitative’ versus ‘qualitative’ defenses, depending on their inherent growth rate or apparency to enemies. Plants may also allocate resources to different defense strategies, such as resistance versus tolerance (Fineblum and Rausher 1995; Fornoni et al. 2003; Mauricio et al. 1997; Stowe 1998; Tiffin 2000), or constitutive versus induced defenses (Adler and Karban 1994; Agrawal et al. 1999; Brody and Karban 1992; English-Loeb et al. 1998; Gianoli 2002).

The selection pressures associated with biotic interactions are also quite variable. Plants are involved in mutualistic interactions with pollinators, seed/fruit dispersers, and mycorrhizae. They are also attacked by a multitude of enemies, including vertebrate and invertebrate herbivores, bacterial and fungal pathogens, leaf and root feeders, tissues-chewers and phloem-suckers. Under the principal of optimal defense, plants must evolve a strategy of allocation to different components of defense that will given them highest fitness, given the impact of the enemies it is most likely to encounter. In order to properly understand how plant defense adapts to an enemy, we must understand both how enemy damage impacts plant fitness, the arsenal of plant defenses and how they impact individual enemies and their population densities, and how these interactions play themselves out in ecological and evolutionary time.

The context of multiple enemies poses additional challenges. The amount of enemy damage or its combined effect on plant fitness may be nonadditive when enemies act in each others presence (criteria 3 in Stinchcombe and Rausher 2001). The effect of a defense trait upon a given enemy may be dependent on the presence or absence of another enemy, *i.e.*, a defense trait my be express a genotype-by-environment interaction, in which the environmental variable is the presence of an additional enemy (criteria 2 in Stinchcombe and Rausher 2001). Finally, plant defense traits may also impact more than one enemy, *i.e.*, there may be genetic correlations in resistance to different enemies (criteria 1 in Stinchcombe and Rausher 2001).

The present works focuses on this later aspect: how defense traits impact different enemies and what characteristics might unite members if an enemy suite. Incorporating the context of multiple enemies into plant-enemy studies will enlighten our understanding of the nature of plant defense, of coevolution between plants and their enemies, of the ecological constraints imposed on the evolution of plant defense, and hopefully provide insights for breeding agricultural crops.

In Chapter 2, I review the relevant literature on this topic. I outline several hypothetical patterns of enemy suites and discuss which plant traits are likely to impact a broad set of enemies versus a specific enemy suite. There are disparate sets of literature that pertain to this topic, ranging from ecology and quantitative genetics, to traditional pharmacological studies to more modern genomics. I enumerate the relevant fields, how they can contribute to our understanding of the specificity of plant defense, review some of the literature, and draw some conclusions from the available evidence.

Chapters 3 and 4 describe artificial selection experiments I performed to address the general question of specificity of plant resistance, using the model system: rapid cycling *Brassica rapa* (Brassicaceae) and a variety of its associated enemies. In Chapter 3, I describe the use of one of its plant enemies (the fungal pathogen *Alternaria brassicicola*) as the selective agent. Several generations of artificial selection resulted in plants populations that were more resistant to *A. brassicicola*. These were subsequently contrasted with control plants for resistance to additional enemies, in order to estimate correlations in resistance between enemies. In parallel, I attempted to select populations of *B. rapa* using other enemies, namely larvae of two lepidopterans (*Pieris rapae* and *Trichoplusia ni*). These artificial selection experiments did not successfully generate distinct populations. These experiments are described briefly in Appendix A.

These experiments using the outcome of an interaction between the plant and its enemy as the selected character do not consider any particular plant resistance trait. Rather, resistance is treated as a plant box, as any combination of characters (unknown to the observer) that affect the amount of enemy damage. A complementary approach is to artificially select populations for extreme states of a putative defense character. This was the approach followed in the experiment described in Chapter 4. Here I describe an artificial selection of populations of *B. rapa* for divergent anthocyanin expression in vegetative tissues. During selection, plants were chosen merely on the basis of the character state, regardless of its biotic effect. Divergent lines were compared with regard to resistance to a number of enemies.

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