5. Conclusions

Plants have a variety of defense strategies, including a multiplicity of chemical defenses that act upon the behavior or performance of enemies. In my review of the literature (Chapter 2), I observed that quantitative genetics studies find a prevalence of non-significant or positive correlations in resistance to different enemies. Mechanistic evidence suggests that plant defense mechanisms rarely affect a single enemy, rather they seem to have an impact on multiple enemies. In many instances there seem to be positively correlated effects on different enemies, even between taxonomically distant enemies or those of distinct feeding guilds. Less frequently, one observes negatively correlated effects of a plant trait, usually between specialist and generalist enemies. I suggest, and provide some evidence, that plant traits that influence an enemy's behavior (antixenotic effects) have a more specific effect than plant traits that attack basic physiologic features (antibiotic effects). I suggest a combination of phenomenological and reductionist approaches, using both quantitative genetic and ecological studies as well as pharmacological and genetic studies of resistance. The latter approach offers insights as to what the material basis of resistance is and which plant traits follow which pattern of effects upon different enemies. As the diversity of defense mechanisms testifies, the research program of investigating each mechanism's effects upon multiple enemies will be painstaking. As enemies may in fact respond to unexpected plant traits, or to combinations of traits, it is advisable to study the effects of whole plant phenotypes upon enemies. Addressing resistance as a 'black box' more closely approximates how enemies impose natural selection upon the entirety of a

plant's phenotype, and offers insight as to how enemies can shape the evolution of plant defense.

I used this approach by artificially selecting populations of rapid-cycling *Brassica rapa* for increased resistance to the cabbage leaf spot *Alternaria brassicicola* (Chapter 3). After seven generations, selected populations were significantly more resistant to *A. brassicicola* infection than control populations. However, there was no significant difference among treatments in resistance to a number of other *Brassica* enemies, indicating an absence of genetic correlations and suggesting that selection acted on plant mechanisms that have relatively specific effects. The marked disadvantage of following a 'black box' approach is that one is left conjecturing as to what the possible defense mechanisms might be. I found a significant difference among treatments in glucosinolate profile. Although glucosinolates are known to have antifungal effects, this evidence is merely correlative. Furthermore, there was substantial variation among lines within treatments with respect to resistance to *Alternaria* and other enemies, suggesting lines may have evolved along different lines, perhaps because of differences among initial founding populations or subsequent sampling effects.

In a complementary experiment, I artificially selected populations of *B. rapa* for divergent expression of anthocyanins in vegetative tissues (Chapter 4). Anthocyanins have a well characterized role in pigmentation, but their role in defense has barely been explored. I found that populations expressing high and low concentrations of anthocyanins in the stem varied significantly in resistance to leaf damage imposed by a number of enemies. In general, specialized and generalist insect herbivores had opposite responses to

selection treatments. As spectrophotometric assays indicate leaf anthocyanin content did not vary among treatments, differences in resistance are probably not due to direct effects of anthocyanins on enemies, but to changes in the metabolic pathways that incorporate the production of anthocyanin and other flavonoids.

These two experiments illustrate how different experimental approaches can provide different answers and perspectives on the specificity of resistance. Experiments that focus on given mechanisms will inform us about the specificity of those mechanisms, but only empirical experiments using enemies as selective agents will inform us what traits will actually evolve. How often will populations respond to selection pressures by a single enemy by evolving resistance mechanisms specific to that enemy? Obviously the answer will depend upon the available genetic variation in a population. But more interestingly, will that answer depend upon the feeding guild of the enemy, its taxonomy, its level of specialization?

We need more experiments that address these questions and we need to integrate these experiments with our knowledge of defense mechanisms. We have accumulated abundant information to understand the mechanisms of defense, but we must begin to synthesize this information to make it useful for thinking about defense against multiple enemies. I hope to have provided a conceptual framework for thinking about mechanisms of resistance to multiple enemies, and contributed with some relevant experiments.