

**The Overcrowded Stage and the Evolutionary Play:  
Resistance of *Brassica rapa* L. (Brassicaceae) to Multiple Enemies**

A Dissertation Presented  
by

**André Levy Coelho**

to  
The Graduate School  
in Partial fulfillment of the  
Requirements  
for the Degree of

**Doctor of Philosophy**

in

**Ecology and Evolution**  
Stony Brook University

August, 2004

**Stony Brook University**

The Graduate School

**André Levy Martins Coelho**

---

We, the dissertation committee for the above candidate for the  
Doctor of Philosophy degree,  
hereby recommend acceptance of this dissertation.

---

Douglas J. Futuyma, Dissertation Advisor

Distinguished Professor, Department of Ecology and Evolution

---

Manuel T. Lerdau, Chair of Defense

Professor, Department of Ecology and Evolution

---

Daniel E. Dykhuizen

Professor, Department of Ecology and Evolution

---

Robert S. Fritz

Professor, Department of Biology, Vassar College

This dissertation is accepted by the Graduate  
School

---

Dean of the Graduate School

## **Abstract of the Dissertation**

The Overcrowded Stage and the Evolutionary Play:

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

by

**André Levy Martins Coelho**

Doctor of Philosophy

in

Ecology and Evolution

Stony Brook University

2004

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.

## Table of Contents

List of Figures	vi
List of Tables	vii
Ackowledgments	vii
Chapter 1. Introduction	1
Chapter 2. Plant Interactions with Multiple Enemies	6
2.1 Abstract	7
2.2 Prelude	8
2.3 Plotlines: the Focus & the Question	10
2.4 Stage Directions: Hypotheses and Models	18
2.4.1 Taxonomic suites	19
2.4.2 Functional feeding guilds	20
2.4.3 Specialist/Generalist divide	21
2.4.4 Enemy mobility	22
2.4.5 Enemy-free space	23
2.4.6 Local enemy density	24
2.4.7 Local enemy diversity	25
2.4.8 Species-specific defenses	25
2.4.9 Interwoven storylines	26
2.4.10 Mechanistic-based predictions	27
2.5 Stagecraft: Tools and Methods	28
2.5.1 Traditional Quantitative Genetics	29
2.5.2 Measurement of selection gradients	32
2.5.3 Artificial selection experiments	33
2.5.4 Hybrid studies	34
2.5.5 Changes in plant environment	35
2.5.6 Enemy community composition across host species	36
2.5.7 Induced Responses to Herbivory	37
2.5.8 Pharmacological bioassays	39
2.5.9 Genetic evidence	40
2.5.10 Mathematical models	41
2.6 ‘Traffic of our stage’: Evidence for Patterns	42
2.6.1 Correlations in resistance	43
2.6.2 Patterns of abundance	43
2.6.3 Pharmacological evidence	46
2.6.4 Genetic evidence	51
2.7 Curtain Call: Conclusions	53
2.8 References	70

Chapter 3. Correlated responses of rapid cycling <i>Brassica rapa</i> (Brassicaceae) to artificial selection for resistance to <i>Alternaria brassicicola</i> (Deuteromycetes)	94
3.1 Abstract	94
3.2 Introduction	95
3.3 Material and Methods	99
3.3.1 Selection design	101
3.3.2 Glucosinolate Analysis	103
3.3.3 Bioassays	105
3.3.3.1 Lepidoptera	106
3.3.3.2 Flea Beetles	106
3.3.3.3 Cabbage Aphid	107
3.3.3.4 <i>T. ni</i> damage under different levels of plant fertilization	108
3.4. Results	109
3.4.1 Selection design	109
3.4.2 Glucosinolate analysis	110
3.4.3 Bioassays	111
3.5 Discussion	112
3.6 References	131
 Chapter 4. Correlated responses to divergent artificial selection in vegetative anthocyanin expression	136
4.1 Abstract	136
4.2 Introduction	137
4.3.1 Selection design	142
4.3.2 Physiological and phytochemical measurements	143
4.3.3 Bioassays	146
4.3.3.1 Lepidoptera	147
4.3.3.2 Flea Beetles	148
4.3.3.3 Cabbage Aphid	149
4.3.3.4 Leaf Spot Infection	149
4.3.4 Correlations among populations	150
4.4 Results	152
4.4.1 Selection design	152
4.4.2 Physiological and phytochemical measurements	152
4.4.3 Bioassays	153
4.4.3.1 Lepidoptera	153
4.4.3.2 Flea Beetles	154
4.4.3.3 Cabbage Aphid	154
4.4.3.4 Leaf Spot Infection	155
4.4.4 Correlations among populations	155
4.5 Discussion	156
4.6 References	183

Chapter 5. Conclusions	187
Appendix	190

## List of Figures

Fig. 2.1 Functional classification of identified <i>Arabidopsis</i> genes	50
Fig. 3.1 Disease severity scores between lines selected for resistance to <i>Alternaria brassicicola</i>	121
Fig. 3.2 Distribution of disease severity scores caused by <i>A. brassicicola</i>	122
Fig. 3.3 Seed production between selected and control lines	123
Fig. 3.4 Total glucosinolate content of each selection line	124
Fig. 3.5 Glucosinolate profile expression	125
Fig. 3.6 Leaf area damaged by first instar larvae of <i>Pieris rapae</i>	126
Fig. 3.7 Leaf area damaged by first instar larvae of <i>Trichoplusia ni</i>	127
Fig. 3.8 Leaf area damaged by adults of <i>Phyllotreta cruciferae</i>	128
Fig. 3.9 Colony size of <i>Brevicoryne brassicae</i>	129
Fig. 3.10 Leaf area damaged by <i>T. ni</i> under three fertilization treatments	130
Fig. 4.1 Hypocotyl color score between base population and selected lines	168
Fig. 4.2 Frequency of color scores	169
Fig. 4.3 Seed production among lines	170
Fig. 4.4 Estimated leaf and stem anthocyanins concentrations	171
Fig. 4.5 Photosynthetic rates	172
Fig. 4.6 Total glucosinolate content of each selection line	173
Fig. 4.7 Glucosinolate profile of each selection line	174
Fig. 4.8 Leaf area damaged by first instar larvae of <i>P. rapae</i>	175
Fig. 4.9 Leaf area damaged by first instar larvae of <i>T. ni</i>	176
Fig. 4.10 Leaf area damaged by first instar larvae of <i>S. exigua</i>	177
Fig. 4.11 Leaf area damaged by adults of <i>P. cruciferae</i>	178
Fig. 4.12 Colony size of <i>B. brassicae</i>	179
Fig. 4.13 Disease severity score caused by <i>A. brassicicola</i>	180
Fig. 4.14 Distribution of disease severity scores caused by <i>A. brassicicola</i>	181

## List of Tables

Table 2.1 Survey of correlations for resistance to natural enemies	57
Table 2.2 Effects of sinigrin on plant natural enemies	61
Table 2.3 Effects of rutin on several plant natural enemies	61
Table 2.4 Effects of proteinase inhibitors on different plant enemies	62
Table 2.5 Effects of lectins on plant natural enemies	65
Table 3.1 Analysis of disease severity score between lines, after 6 gen.	117
Table 3.2 Analysis of disease severity score between lines, after 7 gen.	117
Table 3.3 Average glucosinolate content	118
Table 3.4 Analysis of total foliar glucosinolate content	118
Table 3.5 Average resistance to different enemies	119
Table 3.6 Analyses of resistance to different enemies	119
Table 3.7 Analysis of resistance to <i>T.ni</i> at 3 different fertilization regimes	120
Table 4.1 Analysis of seed set among lines	163
Table 4.2 Analysis glucosinolate concentration among selection lines	163
Table 4.3 Average foliar glucosinolate content	164
Table 4.4 Average resistance to different enemies	164
Table 4.5 Analysis of resistance to different enemies	165
Table 4.6 Analysis of disease scores	166
Table 4.7 Correlations among damage inflicted by different enemies and anthocyanin content	167
Table A1. Analysis of resistance to third instar <i>Pieris</i> larvae	198
Table A2. Analysis of resistance to third instar <i>Trichoplusia</i> larvae	198
Table A3. Analysis of trichome density among full- and half-sib plants	198
Table A4. Analysis of <i>T. ni</i> damage among full- and half-sib plants	199
Table A5. Analysis of <i>P. rapae</i> damage among full- and half-sib plants	199
Table A6. Analysis of <i>P. rapae</i> damage among control populations and lines selected from greater resistance or susceptibility to <i>Pieris</i> damage	199

## Acknowledgements

A doctoral program is a very personal process and accomplishment that cannot be completed without the contributions and assistance of a great many people at different steps along the way, each in their unique fashion. I certainly could not have concluded my dissertation without the help and support of the following people.

My advisor, Doug Futuyma, was a true mentor. Part of my reasoning in coming to the US to get my degree had to do with escaping the European system, in which one simply follows in the footsteps of an advisor's research. I sought Doug after he taught my Evolution class, because of his evident integrated and broad breadth of knowledge and his exceptional ability to communicate. From the start, he pedagogically facilitated the resolution of my struggle to find a specific topic of research, among my many interests. During difficult periods of my experiments, he helped me think through problems with a clear head. Indeed, Doug always approached advising in a deeply human manner, full of understanding for my personal barriers, but relentless when I was in need of a firm word. Doug has an uncanny eye for pointing out experimental caveats, editorial errors, and conceptual inconsistencies. I learned much from his intellectual example, from experiencing his mind at work and seeing him teach. And I gained much from his friendship.

My other committee members were also very helpful, each in his own way. Manuel Lerdau always gave me a fresh perspective on the direction of my research and on interpretation of results. He was generous to have served as my advisor, while Doug was at Michigan, and I am deeply thankful to him for having taken me under his wing when his hands were already full with several other students. James Thomson, an original committee member who subsequently left Stony Brook for Toronto, was pivotal in helping me in the early stages of thinking of a experimental approach and biological model system. Dan Dykhuizen was kind enough to join my committee midway, always had critical comments, and was an important force in keeping me on track. I am grateful for his insistence on the significance of the results of my 'unfruitful' experiments and on writing them as an appendix to my dissertation. Robert Fritz, my outside committee member, was very helpful in my final stages. His own work was an important influence and source of inspiration. His interest in my experiments, a source of encouragement. His timely reviews of my chapters and his incisive comments were an important contribution.

I must thank the Fulbright Commission that paved the way for my adventure to Stony Brook and the Portuguese Fundação de Ciência e Technologia that rewarded me with a doctoral scholarship. Michael Axelrod and John Klumpp, the greenhouse curators, were extremely helpful in my work and reliable sources of amusement. Dan Gilrein and Meg McGrath, of the Long Island Horticultural Research Laboratory (Riverhead, NY), and many people at the NYS Agricultural Experiment Station (Geneva, NY) assisted my collection of *Brassica* enemies and taught me much about their biology. Jonathan Gershenzon and Michael Reichelt, from the Max Planck Institute for Chemical Ecology (Jena, Germany) were extremely generous in performing the glucosinolate analysis.

In addition to my committee members, I must thank many other members from the Department of Ecology and Evolution. R. Geeta supported me for a couple of years as a Research Assistant, during which I learned molecular techniques and phylogenetic methods. More importantly, she was a supportive and provocative friend, tempting me with new projects and tolerating my extra-curricular ventures. Martha Nolan and Marilyn Pakarklis were a family to me. Isabel Ashton and Thomas Merritt were exceptional friends, with a warm hearts and uninhibited mouths, reliable sources of companionship for having fun and helping me through very dark periods. My cohort members, particularly Luciano Matzkin, Amy Dunham, and Heather Throop and my labmates, particularly Lacey Knowles and Aaron Gassmann, were sources of ideas, goofiness and friendship. I should single out many others, but for the sake of brevity, I will thank everyone in E'n'E who has touched my life (you know who you are) and contributed to an indelible example of what a scientific community should be like.

I must also thank several institutions that helped keep my life in balance. The Plant-Insect Discussion Group and the people that contributed to the discussions, which helped me establish a broad foundation in a field largely new to me. The University Counseling Center for supplying free, quality psychotherapeutic support for the larger part of my stay, without which I might have managed to pull through, but certainly not without understanding as much about myself and graduating with the strength to face the forthcoming challenges. The Social Justice Alliance, the student political organization, which provided a much needed forum of political discussion on campus and an outlet for my political voice. Theatre Three, in Port Jefferson, for teaching me the thespian art, giving me the opportunity to exert it, and introducing me to wonderful and supportive friends.

Finally, I must thank my family for their support. My father and sister, for their love. And my mother, for everything.

## **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 plants 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 give 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 may 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 work focuses on this later aspect: how defense traits impact different enemies and what characteristics might unite members of 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 plant 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.

- Adler, F. R., and R. Karban. 1994. Defended fortresses or moving targets: another model of inducible defenses inspired by military metaphors. *American Naturalist* 144:813-832.
- Agrawal, A. A., P. M. Gorski, and D. W. Tallamy. 1999. Polymorphism in plant defense against herbivory: Constitutive and induced resistance in *Cucumis sativus*. *Journal of Chemical Ecology* 25:2285-2304.
- Brody, A. K., and R. Karban. 1992. Lack of a Tradeoff between Constitutive and Induced Defenses among Varieties of Cotton. *Oikos* 65:301-306.
- Bryant, J. P., F. S. Chapin, and D. R. Klein. 1983. Carbon nutrient Balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40:357-368.
- Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. *Science* 230:895-899.
- English-Loeb, G., R. Karban, and M. A. Walker. 1998. Genotypic variation in constitutive and induced resistance in grapes against spider mite (Acari : Tetranychidae) herbivores. *Environmental Entomology* 27:297-304.

- Feeny, P. 1976. Plant apparency and chemical defense. Recent Advances in Phytochemistry 10:1-40.
- Fineblum, W. L., and M. D. Rausher. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. Nature 377:517-520.
- Fornoni, J., P. L. Valverde, and J. Nunez-Farfán. 2003. Quantitative genetics of plant tolerance and resistance against natural enemies of two natural populations of *Datura stramonium*. Evolutionary Ecology Research 5:1049-1065.
- Gianoli, E. 2002. A phenotypic trade-off between constitutive defenses and induced responses in wheat seedlings. Ecoscience 9:482-488.
- Mauricio, R., M. D. Rausher, and D. S. Burdick. 1997. Variation in the defense strategies of plants: Are resistance and tolerance mutually exclusive? Ecology 78:1301-1311.
- Rhoades, D. F., and R. G. Cates. 1976. Towards a general theory of plant antiherbivore chemistry. Recent Advances in Phytochemistry 10:168-213.
- Stinchcombe, J. R., and M. D. Rausher. 2001. Diffuse selection on resistance to deer herbivory in the ivyleaf morning glory, *Ipomoea hederacea*. American Naturalist 158:376-388.
- Stowe, K. A. 1998. Experimental evolution of resistance in *Brassica rapa*: Correlated response of tolerance in lines selected for glucosinolate content. Evolution 52:703-712.
- Tiffin, P. 2000. Are tolerance, avoidance, and antibiosis evolutionarily and ecologically equivalent responses of plants to herbivores? American Naturalist 155:128-138.