Coevolution of the *Ipomoea-Coleosporium*

Natural Plant-Fungus Pathosystem

by

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology in the Graduate School of Duke University

2010
ABSTRACT

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Abstract

Plants and their pathogens coevolve, with pathogen infection and host resistance acting in evolutionary antagonism of each other. Plant-pathogen coevolution has been shown to effect genetic divergence between populations and species, resulting in localized or specialized interactions between hosts and pathogens. Because most of the studies to date investigating plant-pathogen coevolution have been carried out in managed systems and have focused on pairwise interactions, we know little about three aspects of plant pathosystems in natural settings: 1) the role in nature of the gene-for-gene paradigm for genetic determination of resistance; 2) the relationship of host community diversity and structure, and host-pathogen interaction structure, to the antagonistic coevolutionary process; and 3) the factors which underlie and drive local adaptation and specialization of interactions.

This dissertation constitutes the results of research in which I have begun addressing these aspects in a natural plant-fungus pathosystem comprising multiple host species and a single rust pathogen. I have expanded previous characterization of the genetics of plant resistance in one constituent host species in the system by genetic crosses to characterize the basis of resistance in two additional species, finding support for the expectation that the gene-for-gene paradigm of interaction is important in natural
systems. I conducted a cross-inoculation experiment designed to assess host and pathogen variation in infectivity and resistance, to investigate patterns of community interaction structure, and the role that antagonistic coevolution may play in structuring the communities which compose this pathosystem. In these experiments I found that the coevolutionary interaction in this system leads to genetic divergence and the substantial amount of host and pathogen variation I discovered, but that it tends to preserve one pattern of community interaction structure across communities. I expanded my cross-inoculation experimental design to facilitate analysis of quantitative aspects of pathogenesis by measuring the intensity of infections, to test existing hypotheses concerning local adaptation and specialization in pathosystems. In this analysis I found strong host local adaptation and pathogen local maladaptation for the qualitative interaction trait of infectivity, and I found weak host local maladaptation and pathogen local adaptation for the quantitative interaction trait of aggressiveness. I also found host specialization among pathogens, and specialized resistance among hosts, to be common in this system. In light of these results, I hypothesize that the geographic scale of host-pathogen coevolution in this system is that of the local community, and that differences between host species result in persistent but incomplete host specialization in pathogen races.
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1. Introduction

When Charles Darwin described the image of the tangled bank in his view of the natural world, the meaning of the metaphor he invoked placed focus on two aspects of evolution: biological diversity, and the complex and ubiquitous interdependencies of organisms on other organisms (1859). Research has in the meantime refined our understanding of these interdependencies, especially beginning with work in which the term “coevolution” was coined by Ehrlich and Raven in 1964, in the context of an interaction between plants and insects. Since 1964, the concept of coevolution has experienced debate and clarification, but at its core, the concept remains true to that of Darwin’s metaphor: mutually affective evolution between two or more groups of organisms, leading to the generation of biological diversity, and mediated by complex and specialized interactions (Futuyma and Slatkin 1983, Thompson 1994 & 2005).

A particular realm of coevolution has long held the attention of basic and applied research: the interaction between pathogenic or parasitic organisms and their hosts, in a process of antagonistic coevolution. Among systems studied to develop coevolutionary theory, plant pathosystems have been especially informative. Historically, this in part because the effort to stem the effects of parasites and pathogens on plants in agriculture
constitutes a constant and widespread study of processes analogous to coevolution (Rausher 2001), and in part because the fixed spatiality of plant populations and communities has predisposed them to experimental tractability (Thompson and Burdon 1992, Thompson and Cunningham 2002). Plant pathosystems have hence become central to our understanding of antagonistic coevolution between hosts and pathogens.

Of particular import to the study of coevolution was H. H. Flor’s 1956 characterization of the genetics underlying the interaction between cultivated flax and one of its pathogens, flax rust. Flor discovered that the determination of resistance or susceptibility to infection by a particular pathogen race was determined by one locus in the host interacting with one locus in the pathogen, and his “gene-for-gene” concept was rapidly incorporated into the development of theory describing the evolutionary dynamics of systems whose interactions are antagonistic – indeed, in a review of gene-for-gene systems, Thompson and Burdon (1992) note that the first mathematical model of coevolution was based on the gene-for-gene concept (Mode 1958). Additionally, the time between 1956 and 1980 saw a great deal of empirical work in agricultural systems aimed at detecting gene-for-gene interactions between crop plants and their pathogens (Burdon 1987).
Not surprisingly, the valuable work conducted in managed systems during this time led to resulting models’ and expectations’ being based on what had facilitated them: managed systems. In the 1980s, biologists turned focus toward the ecology and evolution of natural plant pathosystems, documented abundant variation in these systems (Fritz and Simms 1992), and provided a nascent body of literature which has since been used extensively by researchers studying managed systems, beginning with Denno and McClure’s integration of work in both managed and natural systems in 1983. Diversity has been described among the constituents of natural pathosystems (e.g. Bevan et al. 1993a, 1993b, Thrall and Burdon 2001), and ecological effects of host and pathogen diversity have been theoretically treated (Garrett and Mundt 1999) and observed (Wolfe 1985, Mundt 1994, Mitchell et al. 2002).

Also not surprising is the fact that the factors which are relatively un-synthesized between the study of natural and managed plant pathosystems are those factors which are most starkly different between the two settings: the amount and patterning of genetic diversity, and the geographic scale and patterning of that diversity. Without a great deal of conceptual similarity between natural and managed systems along the lines of these factors, we have little on which to base related expectations for the
characteristics of natural systems – to derive these expectations requires more empirical work in natural pathosystems.

It is surprising that the topic of Flor’s seminal research is one which has not yet seen broad empirical treatment in natural systems, and it has been argued that the variable genetic mechanisms of plant-pathogen interactions in nature must be explicated instead of attributed too broadly to “gene-for-gene” bases, lest the predictive and explanatory value of the gene-for-gene concept become obscured (Thompson and Burdon 1992). Such explication is underway, but it remains true that we do not know how applicable to natural systems the gene-for-gene paradigm is. One reason to suspect that the kind of interaction predicted by the gene-for-gene concept is not the kind of interaction we might expect in nature is that variation in natural vs. managed systems is very different. Studies of gene-for-gene interactions have historically been carried out in settings of relatively low genetic diversity: one or few pathogen races infecting hosts of one or few genotypes. However, it has been shown that natural pathosystems harbor considerably more diversity than do pathosystems in agriculture (Denno and McClure 1983), and this represents a key difference between the managed systems on which most of our expectations are based, and the natural systems to which we have applied those expectations.
The role of host species diversity and community structure in determining ecological and evolutionary outcomes in natural pathosystems is also expected to be different between managed and natural systems. Agricultural plots rarely comprise more than one species, if more than one genotype, of plants (but for a noteworthy study of exceptions to this trend and their consequences, see Zhu et al. 2000). Recent work has demonstrated that the presence of multiple host species in a pathosystem can have effects which would not be predicted by models based on single-host pathosystems. Pathogen spillover between host species (Daszak et al. 2000) has been observed to affect patterns of infection when the presence of a particularly susceptible host species allowed its abundant pathogen infection to spill over onto less suitable host species (Power and Mitchell 2004). Host species diversity and rates of interspecies transmission have been theoretically shown to either amplify or ameliorate disease outbreaks depending on whether transmission is density- or frequency-dependent (Dobson 2004). Concerning disease incidence, Root’s resource concentration hypothesis (1973) is very frequently for herbivore systems, and often implicitly for pathogen systems, invoked as the basis for the most common prediction for the effect of increased species diversity on a given association: with increased diversity in members of the association, the intensity of the association on average decreases. These findings are each unique, at the current time.
Whether pathogen spillover, dynamics predicted by the resource concentration hypothesis, widespread specialization, or other mechanisms entirely are what lead to general trends in nature is a major frontier in the study of natural pathosystems.

Whether trends are even able to be meaningfully generalized is itself an assumption to which study is due. The effects of there being multiple host species in a natural pathosystem may themselves be variable: different complements of host species may bring about effects which are unique to those complements, such that the relationship of host species diversity to some aspects of disease dynamics and evolution may not be able to be generalized. In this case, we might expect the structure of interaction with pathogens for each of many host communities to diverge. Conversely, the presence of multiple species may be primarily important, and the identities or characteristics of those species may not be as critical, in the determination of disease dynamics and evolution. In this case, we would expect community interaction structure to be influenced significantly by host diversity, and, ceteris paribus, for communities with similar levels of diversity to have similar interaction structures. We lack substantial information on which to base predictions as to which expectation is more reasonable for natural systems, but the question of how host community diversity and structure affects
disease dynamics is one of compelling interest as anthropogenic effects increasingly influence the amount and pattern of biological diversity in nature.

With great diversity in natural pathosystems comes the possibility of even greater diversity in the potential combinations of host types with pathogen types. And with each level of organization for this diversity, there comes potential for interacting organisms to adapt to particular associations. Most commonly, the levels of this kind of adaptation are host specialization (Futuyma and Moreno 1988; for phytophagous insects, Jaenike 1990), and local adaptation (Kaltz and Shykoff 1998).

Studies of local adaptation or specialization in natural pathosystems are relatively abundant (e.g. Dybdahl and Storfer 2003, Montarry et al. 2005; for reviews see Kaltz and Shykoff 1998, Kawecki and Ebert 2004). Here, it is natural pathosystems in which there exists potential local adaptation and potential specialization that are underrepresented in the empirical literature (but see Sicard et al. 2007). Such natural pathosystems would necessarily comprise multiple host species each with some degree of structured geographic distribution, but once again, the historical precedent is largely focused on agricultural or otherwise single-species and relatively uniform interactions (Anderson and May 1991, Grenfell and Dobson 1995, Dobson 2004).
Though some models account for genetic variation within hosts of one species, we do not expect models’ treatment of single-host genetic variability to automatically apply to variability between different host species. The key difference between single-host-species systems and those with multiple host species is the genetic isolation of one host species from others, so that the presence of multiple host species in a pathosystem subjects its evolution to important considerations. First, reproductively isolated host species evolve with genetic independence, increasing the potential for hosts to diverge in traits relevant to interaction with pathogens: for instance, one host species can evolve resistance independently from other host species. Second, the potential for genetic trade-offs theorized to lead to specialism (Jaenike 1990, Kawecki 1998) is present in a pathosystem which includes host diversity: the results of pathogen adaptation on one host species may represent maladaptation on another host species. Obviously, if we are discussing a multi-host-species pathosystem, the subject pathogen must be to some degree a species-generalist. But between the ends of a continuum of specialization lie most pathogens, and there is need to empirically investigate multi-host pathosystems to see where on the continuum their pathogens lie, and if host specialization is important in the determination of interaction structure.
Local adaptation is similar to specialization in that it involves evolution toward higher fitness in associations at one location vs. another, but differs in that the processes underlying between-location differentiation are driven by factors other than those which drive between-species differentiation. Concerning local adaptation, the important differences between managed and natural systems concern scale: agricultural plots are large and characterized by perfectly uniform density; natural populations are small and variously dense (Burdon et al. 1996). An additional consideration arises because host habitat is patchy, and patches are occupied by multiple host species: each host species is distributed across the landscape to form its own metapopulation, but the populations within it are coincident with the populations of the other species. Coevolution in settings such as this – multiple associations across multiple locations – is the subject of Thompson’s geographic mosaic theory (1994, 1999, 2005), a central tenet of which is that selection affecting coevolutionary interactions is geographically variable. The scale of this geographic variation, and the effects of such variation vs. variation between host species, remains an important topic to explore in the study of natural coevolutionary associations such as those found in pathosystems.

In my dissertation work, I studied a naturally-occurring plant pathosystem which comprises three host species and a single, highly variable rust pathogen. First, I
present results of experiments designed to characterize the genetic basis of resistance in the three host species, confirming previous work on one species in the system that found major-gene resistance and considerable variation in its frequency (Kniskern and Rausher 2006). Second, in a cross-inoculation study I show that there is massive variation among the rust pathogen found throughout the system, and that in each host community the structure of the interaction between host species and pathogen races is the same. I hypothesize that this similarity is the result of frequent and rapid host resistance evolution, and that it suggests a pattern of selection common to the different communities, acting to bring about and maintain one pattern of community interaction structure. Third, I expand the cross-inoculation study to include an additional fitness trait, analyze two traits to determine patterns of local adaptation and specialization in both hosts and pathogens, and make inferences about the roles of variation between host locations and between host species in determining observed patterns of infection. The results of this analysis suggest that metapopulation processes may influence the observed patterns of interaction, consistent with predictions of the geographic mosaic theory of coevolution (Thompson 1999), and with predictions derived from other natural plant pathosystems (Antonovics et al. 1997).
2. Genetics of resistance to the rust fungus

_Coleosporium ipomoeae_ in three species of morning glory (_Ipomoea_)

2.1 Introduction

With a few exceptions, genetic studies documenting gene-for-gene interactions have been conducted in agricultural settings (Keen 1990, Agrios 1997). Indeed, Flor (1956) pioneered the concept using cultivated flax and its common pathogen, flax rust. Based on the success of this model in understanding the evolution of crop pathogens, the gene-for-gene model has been hypothesized to underlie much natural coevolution in plant-pathogen systems (Flor 1971, Keen 1990). However, the extent to which this model describes plant-pathogen systems in nature is unclear, and it is reasonable to expect that important differences between agricultural and natural systems, including the monoculture of cultivation _vs._ the diversity in nature, host population size, or length of coevolutionary association between plant and pathogen species, will translate to important differences in the coevolutionary dynamics in the two settings.

A few pathosystems have been studied to characterize plant-pathogen coevolution in nature. Among these are _Silene latifolia_ and its fungal pathogen
Microbotryum violaceum (e.g. Delmotte et al. 1998), Plantago lanceolata and the blight Phomopsis subordinaria (de Nooij and van Damme 1988), species of Avena infected by crown rust (Puccinia coronata) and stem rust (Puccinia graminis) (Oates et al. 1983), and Cakile maritime infected by Alternaria brassicicola (Thrall et al. 2005). But the natural plant pathosystem which has been the most extensively characterized to the benefit of our understanding natural plant-pathogen interactions, coevolution, and disease dynamics is the Linum marginale – Melampsora lini pathosystem (wild flax and flax rust), a fitting first natural plant pathosystem to be appreciably characterized after Flor’s work in its cultivated counterpart (Burdon and Jarosz 1991, Jarosz and Burdon 1991, Thrall and Burdon 2000, Thrall et al. 2002, Thrall and Burdon 2003). To date, the Australian Linum – Melampsora system is also the single known natural system apparently adherent to the gene-for-gene paradigm (Thompson and Burdon 1992), though studies in Arabidopsis thaliana have at times referred to natural selection on well-characterized R-genes (Mauricio et al. 2003).

The Linum – Melampsora system has been found to exhibit several characteristics consistent with the expectations for evolution under gene-for-gene conditions, including genetic determination of host-pathogen compatibility governed by the interaction of one host locus with one pathogen locus at a time, a hypersensitive response form of
resistance, spatial distribution of pathogen types which is maintained despite pathogens’ being highly mobile, and the apparent absence of a universally-virulent pathogen strain or universally-resistant host population (Thrall et al. 2002, Thrall and Burdon 2002, Thrall and Burdon 2003). The molecular evolution of R-genes conferring pathogen resistance has also been extensively studied in Arabidopsis thaliana (for review see Hammond-Kosack and Jones 1997), although the lack of deliberate focus on natural populations of this system limits the extent to which we can use A. thaliana to develop our understanding of the natural dynamics of gene-for-gene evolution. And beyond these systems, little is known about the relevance of the gene-for-gene model in nature.

However in models and in managed systems, much has been uncovered about the evolutionary dynamics (Sasaki 2000, Bergelson et al. 2001), the molecular bases (Dangl and Jones 2001), and the spatial scale of interaction (Thrall and Burdon 1997, Thrall and Burdon 2002) of resistance and virulence evolution in plants and their enemies since the inception of the gene-for-gene paradigm. In gene-for-gene systems, plants exposed to their pathogens typically exhibit one of two phenotypes: susceptibility, in which the pathogen is not recognized and is able to establish infection; and resistance, in which the plant recognizes the pathogen and activates a resistance response, typically the hypersensitive response, in which plant tissue surrounding the
site of pathogen recognition undergoes programmed cell death to prevent the establishment of infection (Agrios 1988, Goodman and Novacky, 1994).

An outstanding question about the applicability of our agricultural-system-based understanding to natural systems concerns the number of loci involved in any particular host-pathogen interaction. In agricultural settings, pathogens and host plants collected from one site at a particular time typically exhibit single-gene segregation of infectivity and susceptibility (Flor 1971). It is well-known that genetically uniform or identical agricultural plots are often enormous relative to the size of similarly genetically uniform populations in nature, and so the scale of agricultural “sites” (64% of which were larger than 1,000 acres in 2002 [USDA ERS 2007]), may be appreciably different than the scale over which natural plant populations coevolve with their enemies. Because resistant plant lines are typically present in a managed system due to their being developed or chosen and then deployed by managers to enhance production in the face of pathogen attack, the distribution of resistance alleles in agriculture is arguably due exclusively to human intervention. Whether natural populations are similar in that they also segregate for resistance at a single locus is the primary question addressed by the present study, in which we investigate a natural plant-fungus pathosystem to characterize the genetic basis of resistance to infection by a rust pathogen, Coleosporium ipomoeae, in three species
of *Ipomoea*. In one of these *Ipomoea* species, *I. purpurea*, rust infection has been shown to reduce fitness considerably, imposing the type of selection which is expected to drive coevolution in plant-pathogen systems (Kniskern and Rausher, 2006a & 2006b).

We also investigate whether different natural populations of a given species of host segregate for resistance to one pathogen race at the same locus, or whether multiple different resistance specificities to a single pathogen race exist in nature. If the genetic basis of resistance to a particular pathogen race differs between two host populations, then *S₂* individuals from a cross between those populations will segregate separately for resistance. Where resistance does segregate separately, some *S₂* individuals from these crosses will be susceptible to infection by the pathogen race to which both parental populations were resistant. A simple explanation for such a finding would be that resistance in each of the two host populations is conferred by alleles at one locus, but that this locus is different in each of the two populations. If these loci are unlinked, then 1/16 of the *S₂* plants will be homozygous for the susceptible allele at each locus, resulting in these plants’ being susceptible to the subject pathogen race.
2.2 Materials and Methods

2.2.1 Materials used in experiments

*Ipomoea coccinea*, *I. hederacea*, and *I. purpurea* are annual plants commonly found in agricultural field margins in the southeastern United States, and they are commonly infected by the rust fungus *Coleosporium ipomoeae*. In nature where *C. ipomoeae* is present near often co-occurring populations of these species, plants are either infected by the rust, or uninfected and showing signs of gross or microscopic hypersensitive response (Chappell, *pers. obs.*; the hypersensitive response: Goodman and Novacky 1994, Heath 2000). Seeds were collected from populations of these three *Ipomoea* species in North Carolina locations, shown in Figure 1. These seeds were germinated in potting soil at the Duke University greenhouse, and then moved to a growth chamber in which they were watered semi-daily and experienced a 16-hour photoperiod, and corresponding thermal regimen of 16 h at 32° C, 8 hours at 22° C. At plant age 21 days, plants were inoculated with an isolate of *C. ipomoeae* urediospores, the collection of which is outlined below.
Coleosporium ipomoeae is a heteroecious rust pathogen which infects members of Convolvulaceae including Ipomoea as its alternate host in a clonal summer stage of its macrocyclic life cycle (Littlefield 1981). The primary hosts of C. ipomoeae belong to the genus Pinus, and are infected by the rust in spring and early summer (Farr et al. 1989). Urediospores were collected from the field on infected leaves, which were removed from plants and placed in airtight bags for transport. Spores were washed from live pustules into the reservoir of a sprayer with distilled water immediately preceding experimental inoculations.

2.2.2 Crossing design

2.2.2.1 Genetic architecture of resistance to C. ipomoeae

To test the hypothesis that populations of each of the three studied Ipomoea species segregate for resistance at a single biallelic locus, wherein resistance is completely dominant to susceptibility, we crossed plants from pairs of same-species host populations. Each pair comprised one host population that exhibited resistance to a particular pathogen race, and another host population that was susceptible to that race. The genetic architecture of resistance was assessed by means of crosses designed to characterize the difference between the two populations: individuals from each pair of
populations were crossed, and F₁ progeny were selfed to produce a segregating S₂ population. Approximately 120 S₂ individuals were then scored for resistance by inoculating them with the respective race of C. ipomoeae, with resistance being indicated by the hypersensitive response, and susceptibility by the presence of sporulating uredia. We used maximum likelihood to test for deviation from the expected ratio of resistant to susceptible S₂ plants, conducting likelihood-ratio chi-square tests to compare the fit of a model based on our a priori hypothesis for genetic architecture and constrained by our data, against an otherwise similar model unconstrained by our data. Our hypothesis for the genetic architecture of the resistance trait was that the trait is determined by a single biallelic locus, with resistance alleles being completely dominant. Thus our expectation for the ratio of resistant (R-) to susceptible (rr) plants was 3:1.

We crossed four same-species population pairs in this experiment: two pairs of I. purpurea populations (1. host CRG:P, susceptible to pathogen race CRG:P, and host CL:P, resistant to pathogen CRG:P; and, 2. host CRG:P, susceptible to pathogen CRG:P, and host LF:P, resistant to pathogen CRG:P), one pair of I. hederacea populations (host CRG:H, susceptible to pathogen LF:P, and host LF:H, resistant to pathogen LF:P), and one pair of I. coccinea populations (host CRG:C, susceptible to pathogen MO:H, and host MO:C, resistant to pathogen MO:H).
After crossing to produce F<sub>1</sub> progeny, parentals from each resistant host population used were selfed to verify that original crosses were between RR and rr individuals. Where S<sub>1</sub> families from these selfings segregated for resistance, lines derived from parents inferred to be heterozygous were discarded and replaced with families confirmed to be from homozygous parentals.

F<sub>1</sub> individuals were selfed to produce segregating S<sub>2</sub> populations, which were then analyzed to determine the genotypes of the F<sub>1</sub> individuals.

2.2.2.2 Tests of allelism

We crossed pairs of host populations both resistant to a given pathogen race, and confirmed parental plants’ homozygosity for resistance by selfing after conducting our crosses, in the fashion described for our methods of examining the genetic architecture of resistance. An average of 188 S<sub>2</sub> individuals from each of three population crosses were scored for resistance. For each S<sub>2</sub> population, we conducted likelihood ratio tests as in our examination of genetic architecture, with our a priori hypothesis being that if the locus conferring resistance is different between the two populations (and it is unlinked), then we will observe susceptibility in 1/16\textsuperscript{th} of the S<sub>2</sub> plants.
2.2.3 Experimental infections and assessment of plant genotypes

Multiple leaves from each of ten infected plants of the same host species at the same site were collected as a source of inoculum. Immediately preceding experimental inoculation, spores were washed from sporulating uredia on collected leaves using distilled water, and the number of spores per unit volume of the resulting suspension was standardized by dilution using a particle counter.

Soil and experimental plants grown in flats were saturated with distilled water 8 hours prior to the onset of darkness in the growth chamber, and flats were covered with 8” clear plastic domes to elevate humidity and facilitate spore germination. Each flat contained four randomly-placed known susceptible plants used as positive controls. 5 mL of uredinial inoculum suspension per flat was applied via a fine spray to the undersides of leaves. During the period of one to two weeks after inoculation, plants were observed to detect the hypersensitive response (indicating resistance) or the presence of uredia (indicating susceptibility).
2.3 Results

2.3.1 Genetic architecture of resistance

The ratio of resistant to susceptible $S_2$ individuals did not differ significantly from the expected 3:1 in any of the 24 $S_2$ segregating populations we produced (Tables 1 through 4). A Fisher’s exact test (shown in tables for each cross) for independence of $S_2$ populations within each cross did not reject the null hypothesis of homogeneity, allowing us to pool $S_2$ data for conducting likelihood-ratio chi-square tests.

2.3.2 Allelism

None of the 277 $S_2$ plants from our crosses of $I. purpurea$ populations resistant to pathogen race CRG:P showed susceptibility (Table 5), consistent with the hypothesis that resistance to pathogen race CRG:P is determined at the same locus in plant populations CRG:P, CL:P, and Ellis:P, and consistent with previous tests of allelism in the plant species $I. purpurea$ (Kniskern and Rausher 2006b).

However, 11 of the 297 $S_2$ plants from our cross between resistant populations of $I. hederacea$ were susceptible (Table 5), and a likelihood-ratio chi-square test did not reject the hypothesis of observing this result against the hypothesis that susceptible plants
should occur in the segregating $S_2$ population in a proportion of 1/16 ($LR \chi^2=2.3950$, df=1, $p=0.1217$).

### 2.4 Discussion

In our investigation of the genetic basis of resistance to infection by *Coleosporium ipomoeae* in three *Ipomoea* species, we find that for each combination of plant population with pathogen race studied, resistance in plants is determined by a single locus of major effect in which resistance is completely dominant to susceptibility. Our results are consistent with previous work that has demonstrated major-gene resistance to infection by *Coleosporium ipomoeae* in the host species *Ipomoea purpurea* (Kniskern and Rausher 2006b): resistant individuals of *I. coccinea*, *I. hederacea*, and *I. purpurea* all exhibit the hypersensitive response to infection when inoculated with pathogen races to which these plants are resistant, and exhibit the formation of sporulating pathogen uredia when inoculated with pathogen races to which these plants are susceptible. Our tests for allelism of resistance between host populations indicated that the trait is determined by the same locus in *I. purpurea* populations at locations CL and LF, for pathogen races CRG:P, and Ellis:P. This is also consistent with previous work in *I. purpurea*, in which
allelic resistance to one pathogen isolate was found between several host populations (Kniskern and Rausher 2006b).

However, our first characterization of resistance to *C. ipomoeae* in *I. hederacea*, though it found single-locus determination of resistance in each of the tests we conducted, uncovered one pair of *I. hederacea* populations in which resistance to one pathogen race is determined by different loci (Table 5). This result is not unexpected, as host-pathogen associations at different locations experience a degree of genetic isolation, and hence we expect there to be genetic divergence between the host and pathogen populations which compose these associations. The resistance genes which are likely to bring about the type of hypersensitive response we have observed are R-genes, which encode proteins involved in the detection of pathogens or pathogen activity (Ellis *et al.* 2000). Whether the interactions we observe are indeed determined by R-genes will require further characterization of these interactions at the molecular level; however, it is R-genes which have been found to underlie resistance resulting in the hypersensitive response, in several plant pathosystems (in *Arabidopsis*: Bergelson *et al.* 2001; in rice: Song *et al.* 1997; in lettuce: Meyers *et al.* 1998; in tomato: Parniske *et al.* 1997). One important aspect of what is known concerning R-genes in these settings is that R-gene
loci occur in several unlinked clusters, each containing many tandemly repeated R-genes (Meyers et al. 2003).

If R-loci are numerous in Ipomoea species as we expect them to be from studies in other plant species, and resistance to infection from a given pathogen race can be the result of any of these loci harboring an allele encoding the necessary recognition specificity, then it is likely that the evolution of resistance to single pathogen races or combinations of races occurs at different loci in different populations. Our detecting such a pattern between two populations of I. hederacea is strong support of this prediction. It is possible that I. hederacea’s resistance evolution is in some way different than that of I. purpurea, or that I. purpurea similarly harbors resistance to various pathogen strains at numerous, however yet unstudied, loci.

We analyzed the data from our test of allelism in I. hederacea to describe possible linkage between the two putative loci underlying resistance to pathogen race CRG:C. The frequency of susceptible $S^2$ individuals was less than the expected 15:1 ratio of resistant to susceptible, and a likelihood ratio chi-square test was positive for deviation from this ratio (LR $\chi^2=4.422$, df=1, $p=0.0354$), suggesting some degree of linkage between the two putative loci. We estimate the rate of recombination between these two loci to be 0.378, by means of maximum likelihood analysis. The likelihood of any
recombination rate \( r \), for our observed numbers of \( S_2 \) individuals’ phenotypes (297 resistant and 11 susceptible), is given by:

\[
\text{(1)} \quad 2 \ln \left( \frac{L_C}{L_U} \right),
\]

where \( L_C \), the likelihood of observing our data for a given value of \( r \), is

\[
\left( \frac{3}{4} r^2 \right)^{297} \left( \frac{1}{4} r^2 \right)^{11}
\]

and

where \( L_U \), the likelihood of observing our data for \( r=0.5 \), reflecting the assumption that the two resistance loci in the two different populations are completely unlinked, is

\[
(R)^{297} (S)^{11}
\]

With \( R \) and \( S \) being the observed frequencies of resistant and susceptible \( S_2 \) individuals, respectively (\( R = 297/308 = 0.9643 \), and \( S = 11/308 = 0.0357 \)). Solving the first derivative of equation (1) for zero, we find the most likely value of \( r \) to be 0.378 (95% CI: 0.491 – 0.270). The 95% CI is determined by the range of \( r \) values for which the likelihood ratio statistic is less than 3.62, the critical value at which the likelihood ratio chi-square test is significant for a difference between this most likely value of \( r \), and either boundary of the CI.
Our finding resistance to one pathogen race, at two loci between two host populations, indicates that gene-for-gene coevolution between different host-pathogen pairings even within one pathosystem may involve different pairs of genes. We have shown that in two such pairings between one race of *C. ipomoeae* and one species of host, *I. hederacea*, the two pairings are mediated by different loci in the host. If it is common in nature that numerous different gene-for-gene interactions can be found across the geographic range of a single pathosystem, then the potential ability of gene-for-gene coevolution to generate biological diversity is enormous. Further, if gene-for-gene coevolution results in genetic divergence between races or populations, leading to geographic variation among host-pathogen associations, then it may play an important part in composing the geographic mosaic.
3. Coevolution leads to genetic divergence but convergence in community structure.

3.1 Introduction

Plants and their pathogens coevolve, with pathogen infectivity selecting for plant resistance, and resistance subsequently selecting for infectivity (Janzen 1980). Evidence for such coevolution has accumulated for a few natural plant-pathogen systems (Thrall et al. 2001, Thrall et al. 2002; Barrett et al. 2007), and indicates that coevolution causes populations, and thus the communities they compose, to diverge genetically as different virulence and resistance alleles arise and are selected for in different populations (Michelmore and Meyers 1998, Dodds et al. 2006). This expectation has been repeatedly confirmed experimentally by investigations that demonstrate, for example, that either plants (Parker 1989, Kaltz et al. 1999) or their pathogens (Parker 1985, Parker 1991, Thrall et al. 2001), are locally adapted to populations with which they interact.

Because most theoretical and empirical investigations of plant-pathogen coevolution have concentrated on a single pathogen species attacking a single host species, little is known about the patterns generated by multi-species coevolution. In particular, coevolution in such systems has the potential to influence community
structure (Augspurger 1988, Packer and Clay 2000). In a single pathogen-multiple host system, one relevant aspect of community structure is the interaction structure, which is determined by the degree of host specialization of pathogen genotypes. At one extreme, one or a few generalist pathogen genotypes may successfully infect all host species present at a particular site. At the opposite extreme, each host species may be successfully infected by a different set of highly specialized pathogen genotypes. While theoretically either of these outcomes is possible for a local community (Regoes et al. 2000), there are no a priori expectations regarding whether genetically diverging coevolving communities will converge to the same pattern of interaction structure. The observation of such convergence, however, would suggest that coevolutionary divergence is constrained to maintain similar interaction structure.

Because previous investigations have focused on interactions between a single pathogen species and a single host species, it is unclear whether in multi-host communities genetic divergence is accompanied by divergence in community structure, or whether the process of coevolution preserves community structure. We report here the first investigation of the effect of coevolutionary divergence on community structure. We demonstrate that while coevolution between the rust pathogen Coleosporium ipomoeae and three species of hosts in the genus Ipomoea causes extensive genetic divergence
between communities, the structure of the interaction is preserved. This pattern appears
to result from the ability of the host plants to stay ahead in the coevolutionary arms race.

3.2 Materials and Methods

3.2.1 The Ipomoea-Coleosporium pathosystem

Throughout the eastern United States, a rust pathogen, Coleosporium ipomoeae, attacks several species of morning glory hosts, including Ipomoea coccinea L., I. hederacea Jacq., and I. purpurea (L.) Roth. The pathogen is a heteroecious rust, which grows on pines (especially Pinus taeda in the southeastern United States) during spring, where it undergoes meiosis, mating and karyogamy. In the early summer, asexual spores colonize morning glories, where the pathogen can undergo as many as 14 asexual generations before producing spores that recolonize pine.

Surveys of communities in North and South Carolina where morning glories are present indicate that they typically contain two or three Ipomoea species (Table 6). Among these communities, there is abundant variation in the distribution of rust infection: both the number of species, and the combinations of which species are infected, varies between communities. However, within each community, the pattern of
infection was constant over three successive summers. Moreover, in most communities with multiple host species present, more than one species is infected.

One possible explanation for this pattern is that the pathogen consists of three cryptic species, each specializing on one of the host species, and that absence of infection on a particular host at a particular site simply represents absence of the associated pathogen strain. To examine this possibility, we performed a phylogenetic reconstruction of pathogen samples from different hosts and sites using two gene regions: an approximately 1400 bp sequence of ribosomal DNA spanning the ITS-2 region, the 5.8S subunit, and the 28S subunit; and an approximately 1800 bp sequence of 18S rDNA. The most likely tree recovered appears in Figure 5. Despite the trees' being imperfectly resolved, the likelihood associated with this tree is significantly higher than that for the best tree in which samples are constrained to group according to host species ($\Lambda = 2 \times \text{difference in log-likelihood} = 142.46, \df = 1, P < 0.001$). This result, coupled with the ability of inocula collected from one host species to infect the other host species (see below), indicates that C. ipomoeae on the different hosts represents a single coevolving species rather than a set of cryptic host species.

To investigate the pattern of host specificity for pathogen genotypes we performed a series of cross-inoculations in the laboratory between pathogens collected
from a single host species at a particular site, and host plants of that and other species
collected at the same and different sites (Figure 2). The set of inoculations performed
represented a compromise between complete coverage of all hosts and pathogens at a
given site and coverage of as many sites as possible. Because individual inocula were
collected from multiple plants of the same species in the field, they may represent more
than one pathogen genotype.

3.2.2 Field Censuses

Morning glory communities were censused to determine the natural distribution
of infection during 2005 – 2009. Several communities were chosen for focused
investigation, reflected in Figure 2. Morning glory communities were visited and
examined during the early summers of 2005-2009. The *Ipomoea* species which were
present in communities each year was recorded after conditions had led to *Ipomoea*
germination. Which of these *Ipomoea* species harbored natural infection was also
recorded. Qualitative assessment of infection's presence/absence was made based on the
observation that between the months of June and August of each year, when *C. ipomoeae*
appeared and spread in a consistent fashion. By late August, individual populations fell
into two discrete categories: greater than 90% of plants infected, or fewer than 10% of
plants infected. At this time, we designated the status of all populations occurring in the
field as infected or uninfected according to these two categories.

3.2.3 Phylogenetic analysis of rust rDNA

Fungal tissue was collected from infected plants in the field at the five locations
referenced in Table 1: CB, CL, CRG, LF, and MO. Sampling was carried out so that fungi
from ten different host plants of each species, from each of these five locations, would be
represented in our collection. Host plants chosen for collection were no less than three
meters from each other, to prevent redundant sampling from one plant. Single live
pustules were excised from live host tissue. Because uredinial pustules reproduce
clonally, each sample represents one fungal genotype. Samples were flash frozen in
1.5mL microcentrifuge tubes over liquid nitrogen, and homogenized by grinding with
plastic pestles. DNA was extracted in CTAB buffer using a protocol for extraction of
dNA from plant tissue (Doyle and Doyle 1990). Using primers Rust2inv (Aime 2006)
and LR6 (Vilgalys and Hester 1990), 15 μL polymerase chain reactions were carried out
to amplify a ca. 1400 bp region of rDNA spanning the ITS-2 region, 5.8S subunit, and 28S
subunit. The ca. 1800 bp 18S rDNA was amplified using primers Rust18S-R (Aime 2006)
and NS1 (White et al. 1990). Thermal cycler conditions for amplification were: 2 minutes
at 94° C; 35 cycles of 0.5 minutes at 94° C, 1 minute at 57° C, and 1.5 minutes at 72° C;
and 7 minutes at 72° C. Using 0.1μL each of 10μM sequencing primers Rust2inv, LR6, LR0R (Moncalvo et al. 1995), and LR3 (Vilgalys and Hester 1990) for the ITS-2 to 28S region, and NS1, NS4, NS5 (White et al. 1990), and Rust18S-R for the 18S subunit, 0.3 μL of each amplicon was sequenced using BigDye Terminator enzyme. Sequencing thermal cycler conditions were: 2 minutes at 94° C; and then 35 cycles of 94° C for 0.5 minutes, 50° C for 0.25 minutes, and 65° C for 4 minutes.

Sequence data were edited using Sequencher® 4.7 (Bromberg et al. 1995), and then concatenated and aligned by eye in MacClade 4.08 (Maddison and Maddison 2001). Four instances of apparent polymorphism within samples were treated as uncertainty.

We selected Coleosporium asterum and C. tussilaginis to represent the outgroup in this analysis. Like C. ipomoeae, both of these species are heteroecious and macrocyclic, infecting pines as primary hosts, and members of Asteraceae as alternate hosts. Sequences for outgroup taxa were retrieved from Genbank. Trees were described using a maximum likelihood search bootstrapped 1000 times in RAxML-HPC (Stamatakis et al. 2005) using the GTRGAMMA model of substitution.

By comparing constrained tree topologies to the most likely topology found above, we tested two hypotheses: 1) if significant host-specialization of pathogen races occurs across locations and leads to isolation of species-specific races, then fungal
samples from each host species should topologically group together; 2) if significant local adaptation of pathogen races occurs across host species and leads to isolation of races at different locations, fungal samples from each location (or locations which are relatively near each other) should topologically group together. Tests of these qualitative hypotheses were carried out through likelihood-ratio comparisons of topologically-constrained trees to unconstrained trees. Differences between constrained and unconstrained tree likelihoods were doubled to compute a likelihood ratio D statistic for each comparison, and the results were used to conduct Chi-square tests for goodness-of-fit with one degree of freedom.

### 3.2.4 Experimental assessment of compatibility

Seeds from mature plants were collected in August-September of years 2006-2008, haphazardly and with 3 meters between collections to avoid repeated collection from single plants. Assessment of compatibility for collected seeds was carried out in the year following collection, such that the rust encountered by experimental plants was that which these plants would have encountered after germinating one year after seed dispersal.

Two field locations (CRG and LF) were chosen for complete reciprocal cross-inoculation, and three locations (CB, CL, and MO) were chosen for additional cross-
inoculations. Because not all host-pathogen combinations from these additional locations could be tested due to space limitations, a subset of combinations was chosen at random, reflected in Figure 2. Each cross-inoculation represents the combination of plants from one host population (one *Ipomoea* species at one location) with spore inoculum collected from one host population. An average of 12 plants were used as experimental hosts for each inoculation, several of which were repeated during each of the years inoculations were carried out. Plants used in controlled inoculation experiments were grown for 14 days in the Duke University Greenhouse in fertilized soil (14-14-14) and were watered semi-daily. Experimental plants were randomly placed into blocks of 36, and grown in identical 36-pot cell packs, each in one greenhouse tray. At a plant age of 14 days, plants were moved to a climate-controlled growth room with a 16-hour photoperiod, and corresponding thermal regimen of 16h at 32° C, 8 hours at 22° C. At 21 days, each 36-plant group was administered an inoculum consisting of a collection of urediospores from one host species at one site.

Urediospores were collected from the field on infected leaves, which were removed from plants and placed in airtight bags for transport. In the laboratory, spores were washed from live pustules with distilled water, and the resulting spore suspension was diluted to a standard 2000 particles/mL. Controlled inoculation was carried out by
first saturating soil and plants with water 8 hours prior to the end of the light stage of the photoperiod. An 8” clear dome was placed over plant trays at that time to elevate relative humidity and simulate natural conditions in the field at dusk. 5 mL of a standardized spore suspension was then applied via a spray bottle to the undersides of experimental plant leaves and the plants were left undisturbed for 7 days before domes were removed. Plants were observed daily from age 28 days to 35 days for scoring; plants on which orange uredia appeared were scored as infected. Plants on which the hypersensitive response, indicated by the appearance of black flecks or spots on leaves, was observed were scored as resistant.

### 3.3 Results and Discussion

Inoculations resulted in one of two outcomes: either a plant became infected, as indicated by the presence of sporulating uredia, or it resisted infection, as indicated by lack of uredia and the presence of small regions of necrotic tissue resulting from a hypersensitive response. Typically, all plants representing a particular host-site combination exhibited one response or the other to a particular inoculum (Figure 6), allowing each host-site combination to be unambiguously characterized as either resistant or susceptible to a particular inoculum. We have shown that the genetic basis
for these interactions is a single biallelic locus of major effect (see chapter 2), and this all-
or-nothing resistance response is typical of gene-for-gene interactions (Keen 1990).

Comparison of patterns of infectivity across all hosts for different inocula reveals extensive genetic variation both within the pathogen and within each host species (Figure 3). Among the 12 inocula tested, only two have patterns of infectivity that could not be distinguished. Similarly, within I. hederacea, I. purpurea, and I. coccinea, respectively, 4 of 5, 4 of 4, and 4 of 4 populations exhibited distinct patterns of infectivity across pathogen inocula. This variation indicates that populations of hosts and pathogen have diverged genetically among sites, as expected.

The set of 100 cross-inoculations can be broken down into four categories (Figure 4): (1) inoculation of the same host at the same site from which the inoculum was collected; (2) inoculation of the same host from a different site; (3) inoculation of a different host at the same site; and (4) inoculation of a different host from a different site.

Infection frequencies were higher for inoculation of the same host than for inoculation of different hosts, regardless of whether they were from the same or different sites. In addition, while there was a substantial probability (0.5) of infection for inoculation of different hosts at different sites, no infections occurred for different hosts at the same site from which the inoculum was collected. Moreover, this pattern appears
to hold for all three host plants: in a 3-way G-test with factors host species, success of infection, and host site (same as or different from site from which inoculum was collected), the interaction between host site and infection success was highly significant ($G = 21.96$, $df = 1$, $P < 0.0001$), while neither the three-way interaction ($G = 0$, $df = 2$, $P > 0.9$), nor the other two-way interactions (host species x host site: $G = 4.69$, $df=2$, $P > 0.05$; infection success x host species: $G = 0.30$, $df = 2$, $P > 0.5$) were statistically significant.

Thus, while inocula from each host species are able to infect other host species from other locations, they are unable to infect other host species from the same location.

These results indicate that the communities that have been examined have converged to a similar interaction structure: to each host that is infected in nature there corresponds a collection of one or more specialist pathogen genotypes that are unable to infect the other hosts at that site. Moreover, it appears this specialization has evolved in situ at each site independently, and results from a unique combination of pathogen and host genotypes at each site. For example, pathogens from the LF site, which are highly host-specific on hosts from their own site, would be host-generalists at the CRG site: on average, inocula from LF would successfully infect 2.7 of the three host species at CRG (Figure 2). Similarly, inocula from MO would minimally infect 2.0 of the three host species at CRG. This is also the average over all the inoculations performed. Similarly,
if host plants were moved between communities, on average they would be susceptible
to twice as many pathogen genotypes as at their native site.

One *caveat* to this conclusion is the possibility that inocula contain multiple
genotypes, some of which are able to infect alternate hosts at different sites and some of
which are not. In this case, the average number of hosts infected at different sites per
pathogen genotype would be less than 2.0, but still larger than 1.0. In other words, the
existence of multiple pathogen genotypes per inoculum does not affect the conclusion
that on average, local coevolution leads to greater host specificity.

We suspect that the apparent equilibrium for interaction structure is a dynamic
one. Rust spores can be carried for long distances by wind, and it is therefore likely that,
periodically, novel genotypes will invade a local community. On average these new
genotypes will have broader host specificities than those genotypes present, but
subsequent evolutionary change results in a reduction of number of hosts infected by
that genotype to one. The novel genotype will then potentially be in competition with
older genotypes that infect that host, and either competitive exclusion or stochastic loss
will occasionally cause the new genotype to replace one or more of the old ones. This
would result in genotypic turnover but a constant interaction structure.
One unanswered question is the nature of the process that leads to narrowing of host ranges of pathogen genotypes. This could occur through evolution of resistance in the host plants, through evolution of avirulence to some hosts by the pathogen, or some combination of both. We suspect that evolution of resistance is more important for two reasons. First, the detrimental effect of \textit{C. ipomoeae} on host fitness is substantial (Kniskern and Rausher 2006a) and would generate selection for resistance. In addition, the abundance of genetic variation for resistance documented here suggests that mutations conferring resistance are not infrequent. Second, the evolution of resistance accounts for the absence of infection of some hosts at some sites (Table 6): once a host has evolved resistance to all local genotypes, no further infection is possible until a virulent pathogen genotype arises either through emigration or mutation. By contrast, evolution by a pathogen of avirulence on a host it currently infects seems less likely because a spore landing on a non-host is effectively dead. Colonization of morning glories in the early summer occurs by a "rain" of aeciospores from pine trees, which means that the probability of any particular spore’s landing on a particular host species is roughly proportional to the abundance of that host species in the community. For genotypes that are able to infect only one host species, there is a high probability of landing on a plant that they cannot infect. Mortality is thus greatly increased in
genotypes with narrower host ranges. In order for greater specificity to evolve, this reduction in mortality would have to be more than offset by large costs of virulence, which in general have not been detected in fungal pathogens (Jarosz and Davelos 1995). Moreover, the common presence in C. ipomoeae strains of virulence to hosts not encountered locally (Figure 2) suggests that costs of virulence are minimal.

While there is little theoretical guidance for expectations about the evolution of host specificity (Thrall et al. 2007) in pathogens, in the rust Melampsora lini there appears to be selection for virulence across a broad spectrum of resistance genotypes in its host Linum marginale (Thrall et al. 2002). This observation has led to the prediction that in communities with multiple host species, selection should similarly favor pathogen genotypes with a broad host range (Thrall et al. 2002). However, the interaction structure of a pathogen-host community is determined not only by the pattern of selection on the pathogen, but also by selection on and evolution of the host species. Although selection for a broad host range may be occurring in the C. ipomoeae-Ipomoea system, our results suggest that this selection has less of an effect on community structure than the evolution of resistance in the host plants. In this system, plants seem to be winning the evolutionary arms race.
4. Local adaptation and specialization in the *Ipomoea-Coleosporium* pathosystem.

4.1 Introduction

Existing models of pairwise antagonistic coevolution that have clarified our understanding of evolution in pathosystems require additional development to describe coevolution occurring at the metapopulation level, and coevolution occurring between a generalist pathogen and its multiple host species. Some models of coevolution in pathosystems have been analogized to arms races: mutually antagonizing partners of a coevolving system engage in reciprocal escalation of their means to attack or defend. In cases of coevolution between a pathogen or parasite and the one host it attacks, this analogy has proven accurate. Who is “ahead” in the arms race is a function of temporal cycles of escalation, and relative rates of adaptation in host and pathogen. However, in cases of non-uniform distribution of hosts (as in metapopulations), or when pathogens attack multiple species of reproductively isolated hosts (as all generalist pathogens do), the coevolutionary interaction itself is non-uniform across the range over which it occurs. In these cases, temporal cycling of advantage due to reciprocal escalation is not enough to explain observed patterns of host/pathogen advantage in space and time. The
expectation that a coevolutionary interaction will vary across its geographic range is well-documented (Thompson 1994), and it has frequently been confirmed that at the local level, antagonistic coevolution takes the form of an arms race (Stahl and Bishop 2000, Hochberg and Holt 2002). But between experiments which have done well to characterize pairwise antagonistic coevolution in systems spanning appreciable taxonomic breadth (for review highlighting several plant-enemy systems, see Rausher 2001), and theoretical work which describes complex coevolution between numerous parties at expansive geographic scale (Thompson 1994, 1999), there is a conspicuous lack of empirical work.

We are beginning to understand the dynamics which result from antagonistic coevolution in which at least one party is distributed across a metapopulation (Thrall et al. 2007). The importance of geographic scale, and of the degree of spatiality of hosts and their enemies, has been emphasized in several studies of coevolution in natural systems. From studies of natural metapopulations, we know that migration and colonization/extinction rates play critical roles in local adaptation of hosts and/or pathogens: when pathogen migration is appreciably greater than that of hosts, pathogens are expected to have an evolutionary advantage and become locally adapted to infect their sympatric hosts (Thrall et al. 2003). Rarely, the opposite has been found,
where rates are greater in hosts than in pathogens, leading to pathogen local
maladaptation (e.g. Kaltz et al. 1999, and see chapter 3). The predominant conventional
wisdom is that pathogens should be locally adapted due to their rapid generation time,
and greater numbers, relative to their respective hosts (Kaltz and Shykoff 1998). Not
surprisingly, the pathosystems for which this wisdom holds true are often those in
which pathogens reproduce more rapidly, and exist in greater numbers, than do
respective hosts. Where pathogen generation time is slower than that of hosts, or where
effective pathogen numbers are significantly lower than those of hosts, we simply expect
hosts to be locally adapted based on the same reasoning of our conventional wisdom:
rapid generation time and great numbers translate into evolutionary potential, and we
expect the party with greater evolutionary potential to have the advantage in
antagonistic coevolution. Where relative generation time and/or population sizes fail to
explain observed patterns of local adaptation, additional factors underlying
evolutionary potential have been investigated: genomic complexity in a paramecium-
bacterium pathosystem (Adiba et al. 2010); and migration, selfing vs. sexual
reproduction, and differential effects of drift in a plant-fungus pathosystem (Kaltz et al.
1999).
A system in which multiple host species co-occur and serve as hosts to a single pathogen species will not necessarily adhere to our expectations for the coevolutionary dynamics of single-host systems. First, the definition of local adaptation may change in multi-host systems. Traditionally, local adaptation has been measured in one of two ways, and a pathogen strain is deemed locally adapted if: 1) its performance represented by some metric of pathogenesis is greater on sympatric than on allopatric hosts; or, 2) its performance on sympatric hosts is greater than the performance of allopatric pathogen strains on the same hosts. The two traits which are commonly investigated in local adaptation studies are infectivity (the frequency with which a pathogen is able to establish infection on a given host background, where more individuals infected equals higher infectivity), and aggressiveness (the intensity of a pathogen’s infection on a given host, typically represented by propagule production rate, fitness cost to the host, or area of infection). In a multi-host system, a given location may include hosts of multiple species, such that a pathogen could become locally adapted to a community of hosts, or a locally adapted and species-specific pathogen could become adapted to a single-species population at a given location. We have little data on which to base a priori expectations for the patterns of adaptation that should emerge in a multi-species pathosystem. If pathogens have more evolutionary potential than their hosts to adapt,
should they become locally adapted, host-specialized, both, or neither? And if hosts
have the greater evolutionary potential, what pattern of pathogen local maladaptation
should we expect?

Important considerations for these questions are the fact that infectivity and
aggressiveness are different traits, each actually a description of a host-pathogen
interaction instead of a host or pathogen itself, and that evolutionary potential to affect
these two traits may be different in hosts and pathogens (Dybdahl and Storfer 2003). For
example, pathogens may have great evolutionary potential for traits describing the
intensity of infection: genetic variation for pathogen growth rate, and selection for
optimal pathogen growth rate, may be greater in pathogens than corresponding
variation and selection are in hosts. Accordingly, pathogen evolution may often be
primarily responsible for determining the aggressiveness of a given host-pathogen
interaction. In the case of gene-for-gene coevolution, plants have numerous loci which
are capable of encoding resistance specificities against numerous pathogens, and
resistance is the result of the pathogen being detected (for reviews see Keen 1990, Jones
and Dangl 2006). Any one of these many loci is capable of harboring an allele conferring
resistance to a novel pathogen, and the pathogen product or process which triggers the
resistance response is thought to often be essential to pathogenesis. Here, the
evolutionary potential of a host to evolve resistance may be great, where the evolutionary potential for the pathogen to regain the ability to infect a resistant host may be limited by the pathogen’s potential to alter a pleiotropically constrained, essential process or pathway. Accordingly, host evolution may often be primarily responsible for determining the infectivity of a given host-pathogen interaction.

These considerations lead us to testable hypotheses. If our investigation is at adequate spatial scale to differentiate localities, we expect a trend toward local adaptation in traits which have relatively high evolutionary potential. In a system where host evolutionary potential to affect infectivity exceeds that of pathogens, we expect hosts to be locally adapted, such that they have relative advantage against local versus distant pathogens. Where pathogen evolutionary potential to affect aggressiveness exceeds that of hosts, we expect pathogen aggressiveness to be optimal on local hosts. In systems where pathogens do not, or only rarely, cause mortality of hosts, higher aggressiveness equates to higher fitness, because aggressiveness does not limit transmission by killing hosts.

We set out to test these hypotheses in a natural plant-fungus pathosystem which consists of three host species, each distributed over a metapopulation, and a single rust pathogen found to commonly infect each of the host species. We characterized the
pathogenesis interaction of several combinations of pathogen race with host population, to make inferences about local adaptation and specialization in both hosts and pathogens, affecting traits which determine the probability and intensity of infection.

4.2 Materials and Methods

4.2.1 The pathosystem

*Ipomoea coccinea* L., *I. hederacea* Jacq., and *I. purpurea* (L.) Roth are among the alternate hosts infected by the rust pathogen *Coleosporium ipomoeae* during the summer uredinial stage of the rust. These three morning glory species are common in agricultural field margins and other disturbed habitat in the southeastern United States, and commonly co-occur at sites throughout their overlapping ranges. Generally, *I. hederacea* germinates and flowers earlier (by 1-2 weeks) than does *I. purpurea* (Smith and Rausher, 2007), and *I. coccinea*’s germination and flowering times are indistinguishable from those of *I. hederacea* (Chappell, *pers. obs.*). Outcrossing rates in these species vary: *I. hederacea* is highly selfing (93%, (Ennos 1981)), *I purpurea*’s selfing rate has been reported to be between 65% and 74% (Ennos, 1981; Schoen and Clegg, 1985), and though selfing in *I. coccinea* has not been explicitly quantified, the species is known possesses self-fertility
(Martin 1970). Viable hybrids between these species have not been observed in nature 
(Guries 1978).

These three *Ipomoea* species exhibit resistance to infection from *C. ipomoeae* 
consistent with the expectations for a gene-for-gene system (see chapter 2). Resistance to 
one pathogen genotype is determined by one locus in plants, with resistance being 
completely dominant to susceptibility. Susceptibility is evidenced by the presence of 
bright orange sporulating uredia on the undersides of leaves, and on stems, after the 
rust’s incubation period of 10-14 days. Resistance is evidenced by the absence of uredia, 
and the presence of small areas of dead cells surrounding the site of a germinated fungal 
spore, with the appearance of small black flecks indicative of the hypersensitive 
response (Heath 2000).

The *Ipomoea* plants used in our experiments were grown from seeds collected at 
field sites shown and described in Figure 7 and Figure 8. Seeds were haphazardly 
collected from plants during the fall seasons of 2006, 2007, and 2008 for use in 
experimental infection experiments, with 3 meters between collections to ensure that 
they came from separate individuals. Plants used in controlled inoculation experiments 
were grown for 14 days in the Duke University Greenhouse in fertilized soil (14-14-14), 
and were watered semi-daily. Experimental plants were randomly placed into blocks of
36, and grown in identical 36-pot cell packs, each in one plant propagation tray. At a plant age of 14 days, plants were moved to a climate-controlled growth room with a 16-hour photoperiod, and corresponding thermal regimen of 16h at 32° C, 8 hours at 22° C. At plant age of 21 days, each 36-plant group was administered an inoculum consisting of a collection of urediospores from one host species at one site. Details of inoculum collection are given below.

*Coleosporium ipomoeae* is a needle rust of pine. It is heteroecious, infecting species of pines as its primary host and several species of Convolvulaceae including *Ipomoea* as alternate hosts. The life cycle of *C. ipomoeae* is summarized in Figure 9. In the southeastern United States, infection on pine is commonly observed on *Pinus taeda*, and where communities of *Ipomoea* are found, the present *Ipomoea* species are the only nearby alternate hosts found infected by the rust. While infecting *Ipomoea*, the rust reproduces clonally. Urediospores autoinfect hosts, and are transmitted between different host plants by wind and rain. At summer's end, the repeating clonal stage of the pathogen's life cycle terminates with the production of telia, which produce basidiospores to infect primary hosts. Pines are host to the pathogen when it undergoes sex and karyogamy.

*C. ipomoeae* was collected in uredospore form for use in controlled experiments. Leaves of infected plants at field locations were collected and placed in sealed plastic
bags for transport to the laboratory, where distilled water was used to wash
urediospores from sporulating uredia. The resulting suspension was diluted to 2000
particles/mL, and then used as experimental inoculum.

4.2.2 Infectivity

We challenged experimental plants with field-collected inoculum to assess the
infectivity of host-pathogen combinations. Plants used in infectivity assessments were
grown from seed collected from the field in the fall, and the inoculum delivered to these
plants was collected during the following summer. Thus, plants were challenged with
inoculum they could have naturally encountered in the field (depending on their
location), after germinating in the summer following the time of seed collection. We call
each experimental host-pathogen combination a cross-inoculation, representing the
combination of plants from one host population (one Ipomoea species at one location)
with spore inoculum collected from one host population. Each cross-inoculation
involves 12-36 plants from one population, randomly blocked into separate 36-cell flats
in combination with other plants to be challenged by the same single inoculum.

Soil and plants were saturated with distilled water 8 hours prior to the onset of
darkness in the growth chamber, and flats were covered with 8” clear plastic domes to
maintain humidity and simulate natural field conditions at dusk. For each flat of plants,
5 mL of inoculum was applied *via* a spray bottle to the undersides of leaves, and plants were thereafter bottom-watered, to ensure infections were permitted to proceed undisturbed, and to ensure that no cross-contamination of plants occurred. After inoculation, plants were observed to detect the hypersensitive response (typically evident 2-3 days after inoculation) or the presence of uredia (10-14 days after inoculation). The metric of infectivity in this setting is straightforward: for any defined group of plants which have in common that they were challenged with one source of inoculum, infectivity is the proportion of plants infected. We considered infectivity of each inoculum against plants from its own source population, and against plants grouped by species (e.g. native *vs.* non-native species), location (e.g. sympatric *vs.* allopatric hosts), and combinations of these groupings (e.g. all native allopatric *vs.* all non-native allopatric).

### 4.2.3 Aggressiveness

We assessed pathogen aggressiveness on individual plants by measuring spatial characteristics of infections: proportion of leaf area infected, number of pustules on an infected leaf, and average pustule size. Aggressiveness measurements were taken at 28 days after inoculation. At 14 days after inoculation, we expect all viable infective spores which were able to establish infection to have done so, and to have produced
sporulating pustules. At this point a primary determinant of our metrics will be average spore viability, where inocula with greater spore viability will create apparently more aggressive infection. At 28 days, enough time has passed for one round of autoinfection to have occurred, so that at this time our metric captured a composite of growth rate and average spore viability, refining our measure of aggressiveness to reflect an additional quantitative aspect of pathogenesis.

Measurements themselves were taken by means of standardized digital imagery. 12 megapixel images of infected leaves were captured and analyzed in RGB format. For each leaf, color ranges corresponding to uredia (generally, bright orange), to telia (generally, rust orange/red), and to uninfected leaf tissue (generally, green) were specified by eye. By viewing each leaf image individually, and verifying that color range specification included only uredia, telia, or uninfected leaf tissue as applicable, we controlled for variation in leaf hue due to variable moisture or daylight. We tested the repeatability of this method by repeating color specification several times for individual images; the difference between assessed aggressiveness due to variation in color range specification was most often zero, and never greater than the difference between any two measured leaves from the same experimental inoculation. Variation in pustule size was minimal: pustules occupied 526.95 pixels on average, with a standard deviation of
73.04 pixels. No two inoculation combinations resulted in pustule sizes means which were significantly different. For this reason, leaf area infected and pustule count were highly correlated (R²=0.92, p<0.0001). Because our algorithm determining pustule count was subject to occasional errors when adjacent pustules were counted as one, but the leaf area infected calculation was exempt from this, we chose leaf area infected as our dependent variable in analysis of aggressiveness.

### 4.2.4 Statistical tests of local adaptation and specialization

#### 4.2.4.1 Infectivity

Infectivity data were subjected to nested analysis to investigate the dependence of host-pathogen compatibility response on the relationship of inoculum source host to inoculation target host. The response variable was binary: either infection, or resistance indicated by the hypersensitive response. Sources of inoculum, and targets of inoculation, were identified as populations of host species at single locations – for instance, host species *I. coccinea* at location CB constitutes one such host population, and an isolate of *C. ipomoeae* from this host population is named CB:C (“C” for host species *I. coccinea*). Experimental inoculations were each assigned to one of two treatment categories describing the relationship of inoculum source species to target host species:
intraspecies inoculations, in which source and target hosts were both of one species; or
interspecies inoculations, in which source and target hosts were of different species.
Similar assignment was made to categories describing the relationship of inoculum
source location to target location: sympatric inoculations, in which source and target
hosts from the same location; and allopatric inoculations, in which source and target
hosts were from different locations. Four categories result from this assignment of
inoculations to two subcategories of two levels each, as discussed in chapter 3 and
summarized, for infectivity, in Figure 4.

Analysis was conducted using generalized linear mixed model ANOVA (probit
regression in SAS Proc GLIMMIX, Schabenberger 2007). Our model included as fixed
effects the following variables: 1) species of inoculum source; 2) species of inoculation
target; 3) species relationship of source to target (with two classes, intra- and
interspecies); and 4) location relationship of source to target (again with two classes,
sympatric and allopatric). In this analysis, locations were nested within species, and
treated as a random effect. Degrees of freedom were estimated by the Satterthwaite
approximation.

Because both effects describing inoculation treatments (intra/interspecies and
sym/allopatric) were significant, we conducted a series of planned comparisons to test
individual pathogen races and host populations for local adaptation and/or host specialization (or specialized resistance) indicated by patterns of infectivity. These comparisons allowed us to control for variation among target host species, and for effects of pathogen host local adaptation or specialization, when testing for only one of these factors. In all contrasts, familywise values of $\alpha$ were computed using Šidák’s multiplicative correction.

### 4.2.4.2 Aggressiveness

Aggressiveness data were subjected to similar nested analysis to investigate the dependence of quantitative pathogenesis response on the relationship of inoculum source host to inoculation target host. The measured variable in analyses of aggressiveness was proportion leaf area infected, varying continuously between 0 and 1. Identities of inoculum sources and targets, as well as experimental inoculation categories (intra/interspecies, and sym/allopatric inoculations), were defined in the same way as in the analysis of infectivity data.

The effects of inoculum source and target, and inoculation treatment category, were analyzed by mixed linear model ANOVA (SAS Proc MIXED, Littel et al. 1996). The response variable (proportion of leaf area infected) was arcsine square root transformed, and the fixed and random effects specified in the model were the same as those in the
analysis of infectivity data. Model fit was assessed by an Anderson-Darling test of normality for Studentized residuals, and the normality of residuals could not be rejected ($A^2 = 0.452249$, $p > 0.2500$, Figure 18). We further tested our model specification by a likelihood-ratio test comparing our model to one which included additional covariance parameters due to the specification of additional random effects: the interaction between inoculum source location and target host species, and the interaction between inoculum source species and target host location. The likelihood-ratio test did not reject the hypothesis that the more parameterized model was no more descriptive than the model we chose for our analysis ($LR \chi^2 = 2.4$, $df = 2$, $p = 0.30119$).

Again, both effects describing inoculation treatments (intra/interspecies and sym/allopatric) were significant, and we conducted planned comparisons to test individual pathogen races and host populations for local adaptation and/or host specialization (or specialized quantitative resistance) indicated by patterns of aggressiveness. Denominator degrees of freedom were computing using the Satterthwaite approximation, and in all contrasts, familywise values of $\alpha$ were computed using Šidák’s multiplicative correction.
Because all experimental inoculations involving pathogen race CB:C that were allopatric but against the race’s native host species resulted in resistance, we excluded this race from our analysis of inoculation treatment categories’ effects on aggressiveness.

4.3 Results

4.3.1 Infectivity

The ratio of the generalized $\chi^2$ statistic to its degrees of freedom in our analysis was close to 1 (1.19), supporting our choice of linkage function and model parameters. Covariance parameter estimates for inoculum source location and inoculation target location random effects were 0.4217 (SE=0.2330) for inoculum source location nested within host species, and 0.6653 (SE=0.3042) for inoculation target host location nested within host species.

We found substantial variation in the frequencies of infection for pairings of pathogen races with host populations, and the overall distribution of these frequencies was bimodal with modes near 0 and 1 (as in Chapter 3, Figure 5). Overall infectivity of one pathogen race (MO:P, 91.7%) was higher than the average infectivity of all other races (likelihood ratio $\chi^2=49.133$, df=1, p<.0001). In our mixed model analysis, this difference was found to be marginally significant between the best unbiased linear
predictor for MO:P’s average probit-transformed infectivity with location as a random
effect (BLUP for MO:P: 1.279, SE 0.410, DDF=9.244 F=3.73, p=0.0845). Similarly, one
pathogen race (CRG:P, 36.8%) had lower average infectivity than all other races
(likelihood ratio $\chi^2=100.939$, df=1, p<.0001), and the difference was reflected in a
significant difference between the BLUP for CRG:P’s average infectivity and that for the
average of other races (BLUP for CRG:P: 0.568, SE=0.402, DDF=16.35 F=11.05, p=0.0091).
No additional significant differences between races’ average infectivity vs. that of others’
average were found either in the infectivity data, or in the BLUPs for probit-transformed
infectivity data in our mixed model analysis. In general, both raw data and the
estimates from our analysis show that there is a great deal of variation among pathogen
races for infectivity, but that there appears to be only one outlier race with an
exceptional pattern of infectivity.

Both inoculation treatment effects were significant (intra/interspecies
inoculations: $F=179.11$, df=1, p<.0001; sym/allopatic inoculations: $F=73.16$, df=1, p<.0001).
Effects of inoculum source species ($F=0.07$, df=2, p=0.9374) and inoculation target species
($F=1.80$, df=2, p=0.2176) were not significant. The significance of experimental treatment
in our results indicates a strong effect of the relationships between source and target
populations on infectivity, and constitutes evidence that either host populations,
pathogen races, or some mixture of both, are locally adapted and/or specialized in their interactions. Accordingly, we proceeded to planned contrasts to characterize these patterns.

Contrasts comparing individual pathogen races’ infectivity on sympatric vs. allopatric hosts, as well as on native vs. alien host species, are shown in Table 7 and Table 8. Contrasts comparing sympatric vs. allopatric pathogen races’ infectivity on individual host populations, as well as the infectivity of races from native vs. non-native host species on individual host populations, are shown in Table 9 and Table 10. Relative to our a priori hypotheses, these results are consistent with the prediction that host populations should be locally adapted and/or specialized in traits affecting infectivity, based on predicted evolutionary potential to affect infectivity being greater in hosts than in pathogens.

4.3.2 Aggressiveness

Covariance parameter estimates for pathogen and host location random effects are shown in Table 11. Noteworthy here is that the estimate for inoculum source location nested within source species is high relative to the estimate for inoculation target location nested within target species. This result suggests that each pathogen race’s response to treatment is to some degree parallel, when we control for variation in
host populations as it affects pathogen races’ response to treatment. In other words, a given pathogen race may exhibit high aggressiveness on allopatric host population A, moderate aggressiveness on allopatric host population B, and low aggressiveness on its home population C, but because most pathogen races’ aggressiveness on host populations A, B, and C are relatively symmetrical to this pattern, we are able to make inference about the generalized effect of interaction sym/allopatry on aggressiveness.

Average pathogen race aggressiveness varied between 6.62% (SD 7.32%) for pathogen race MO:P, and 33.03% (SD 8.04%) for pathogen race LF:P. The variation within individual pathogen races’ average aggressiveness for particular combinations with host populations during experimental inoculation was highly variable as well: for example, pathogen race CB:C was very aggressive on experimental hosts from host population CB:C, averaging 43.03% area infected; however, on hosts from population CRG:P, pathogen race CB:C averaged only 2.71% area infected.

Both inoculation treatment effects were significant in the analysis of aggressiveness data (intra/interspecies inoculations: F=329.23, df=1, p<.0001; sym/allopatric inoculations: F=11.70, df=1, p=.0007). And again, effects of inoculum source species (F=0.76, df=2, p=.4991) and inoculation target species (F=1.00, df=2, p=0.4014) were not significant. Contrasts similar to those of the infectivity analysis, but
here comparing individual pathogen races’ aggressiveness on sympatric vs. allopatric hosts, as well as on native vs. non-native host species, are shown in Tables 12 through 15, and the complementary contrasts of aggressiveness for individual host populations are shown in Table 16 and Table 17. Table 15 shows the important case in which pathogen races’ aggressiveness was tested on native host species at home locations vs. away locations: if geographic variation leads to local adaptation in this system, then pathogen races will be locally adapted to their home/native hosts vs. their native hosts at other locations.

Pathogen races’ average aggressiveness on sympatric/native host populations, and on allopatric/native host populations, is shown in Figure 10. Here it can be seen that, though few of the differences are significant, some pathogen races are on average more aggressive when infecting allopatric/native hosts than when infecting sympatric/native hosts. Data for pathogen race CB:C are shown here for reference, though this race was excluded from our planned comparisons to analyze local adaptation and specialization in aggressiveness. Figure 11 and Figure 12 show overall averages of pathogen races’ infectivity on native vs. non-native host species, and aggressiveness on sympatric vs. allopatric hosts.
4.3.3 The Relationship of Infectivity to Aggressiveness

Figure 13 shows the relationship of pathogens’ average infectivity to respective average aggressiveness, across all experimental inoculations conducted. The relationship can be described as a triangular one, with combinations of high infectivity with high aggressiveness being absent from the pathogen races we studied. A similar relationship can be seen in Figure 14, which shows the average aggressiveness of pathogen races on home hosts as a function of the races’ average allopatric infectivity: again, combinations of high allopatric infectivity and high aggressiveness at home are not found among the pathogen races we studied. It is noteworthy here that the observed relationship exists for combinations of pathogen race and host population which are not currently realized in nature, and supports the hypothesis that potential aggressiveness is realized often enough to show some signature of natural selection or constraint as uncovered by our cross-inoculations.

Figure 15 and Figure 16 show infectivity-aggressiveness relationships which appear to lack any pattern. Figure 15 demonstrates that allopatric aggressiveness and allopatric infectivity of pathogen races do not adhere to a triangular relationship, and are not otherwise correlated. Figure 16 shows a similar lack of pattern in the
relationship between pathogen races' aggressiveness on home host populations and races' average infectivity on non-native host populations.

A significant negative correlation was found between pathogen races' average infectivity and average aggressiveness on non-native host populations, shown in Figure 17.

### 4.4 Discussion

Both Coleosporium ipomoeae and the three studied species of Ipomoea hosts are highly variable for traits affecting pathogenesis, in the field. Differences between predicted values of infectivity and aggressiveness for inoculations of different design indicate local adaptation and specialization in pathogen races and host populations. The pattern of this adaptation appears consistent with the hypothesis that the member of the pathosystem with the higher evolutionary potential for a pathogenesis-mediating trait will have an advantage which results in local adaptation and/or specialization relative to other member(s) of the pathosystem (Kawecki and Ebert 2004, Adiba et al. 2010).

The general relationships between aggressiveness and infectivity in pathogen races, and complementarily between aggressiveness-mediating quantitative resistance and specialized resistance in host populations, were both negative.
4.4.1 The model

We chose to investigate variation in infectivity and aggressiveness by means of a generalized linear mixed model, to allow us to specify between-population variation as a simple random effect, and thus examine specifically the effects of our experimental treatments. Our dataset for aggressiveness is necessarily unbalanced, as well. Not all host-pathogen combinations are compatible, such that we cannot compare the aggressiveness of pathogen races against a fixed array of host backgrounds. Every pathogen race is different in its profile of compatibility.

Our choice respects the biology which leads to this necessary imbalance in our aggressiveness data. Infectivity and aggressiveness are known to be determined by different genetic mechanisms in some systems (Cumagun et al. 2004), and expected to be determined by different genetic mechanisms in others (Sicard et al. 2007). This means that the aggressiveness of a pathogen against a host genotype on which the pathogen is not infective is not zero; instead, it is unrealized because infection is precluded by the effects of qualitative resistance which determine infectivity. It is true that unrealized traits are not under natural selection, but in a pathosystem where a coevolutionary arms race results in pathogens’ alternation of ability and inability to infect the hosts with which they coevolve, potential aggressiveness is often realized after evolution of infectivity de novo or by migration. The importance of this realization is evident in the study of novel host-pathogen associations, where the character of interactions is often
extreme or otherwise unexpected (Parker and Gilbert 2004). We do not assume the aggressiveness of incompatible interactions is zero, and are forced to treat respective data as missing. Our simple mixed model was constructed and analyzed using the MIXED procedure of SAS, in which the estimation of unknown parameters describing random effects is carried out using maximum likelihood (Littell et al. 1996). We found this methodology the most conservative one which is, at least theoretically, also able to accommodate aggressiveness’ data missing at random (Rubin 1976, Little 1995).

The primary rationale for our model choice was not its accommodation of contingency; it was to allow us to study local adaptation and specialization. We specified locations as random effects because our sampling of this pathosystem included only a small fraction of the locations over which it is distributed. Recognizing random variation among locations is particularly important because we do not expect all pathogen races or host populations to be locally adapted at all times as the action of coevolutionary antagonism progresses (Dybdahl and Storfer 2003).

4.4.2 Evolutionary Potential

As our understanding the deterministic genetic factors of plant pathogenesis has increased, our theoretical expectations for adaptation in pathosystems have changed (Greischar and Koskella 2007; Hoeksema and Forde 2008). The expectation that pathogen and parasite populations should be locally adapted to infect their home hosts
has been based on the knowledge that most pathogen and parasite organisms have shorter generation times, larger population sizes, and greater potential to migrate, relative to their respective long-lived and relatively stationary hosts (Chaboudez and Burdon 1995, Ebert and Hamilton 1996, Lively and Dybdahl 2000). Not surprisingly, this expectation has informed much of the theoretical treatment to date addressing issues of local adaptation in pathosystems, and we know of only one model of host-pathogen coevolution which generally predicts pathogen local maladaptation (Morgan et al. 2005).

However, empirical work has uncovered several instances of pathogen local maladaptation (Kaltz et al. 1999, Gandon and Michalakis 2002, Lajunesse and Forbes 2002, Adiba et al. 2010). Such studies have identified two factors which have the potential to influence patterns of local adaptation in antagonistically coevolving pathosystems: constraints on potential to evolve adaptively for traits affecting pathogenesis (e.g. Adiba et al. 2010); and metapopulation factors, especially migration (e.g. Thrall et al. 2002). The first of these factors is similar to the basis for expecting pathogen/parasite local adaptation due to parameters such as generation time and population size: characteristics of pathogen and parasite populations which effectively limit the rate of adaptation (e.g. the absence of sex), relative to the potential rate of
adaptation in a host population, are expected to hinder pathogen/parasite local adaptation. One surprising derivative of this expectation is highlighted by Adiba et al. (2010), in which the relatively low genomic complexity of a parasite relative to its host is hypothesized to limit the parasite’s ability to adapt to a local host population. A concrete interpretation of this expectation in the context of plant pathosystems is consistent with the gene-for-gene paradigm: whereas a host population may harbor several R-loci, each of which is capable of harboring an allele conferring resistance to a given pathogen genotype (potentially available by mutation, or by immigration), the pathogen is detected by the product of the effective R-alleles due to the action or presence of a dominant allele at only one pathogen avirulence locus. In a gene-for-gene interaction, for the pathogen to evolve infectivity against a resistant plant genotype requires the unlikely event of change at a single pathogen locus to recessive novelty. The host, however, can evolve completely dominant resistance due to allelic migration or mutation at any one of potentially hundreds of loci.

Conversely, where pathogen evolutionary potential to affect a trait, such as aggressiveness, is high whereas host potential to mitigate it is constrained, we expect pathogen local adaptation. Pathogens that recruit host pathways, exploiting them for resource delivery or propagule production, interact with host loci that will be
pleiotropically linked to other aspects of host fitness, and are thus evolutionary
constrained (Antonovics and Thrall 1994). Empirical work describing a negative
relationship between resistance and other fitness components is abundant (e.g. Bergelson
and Purrington 1996, Thaler et al. 1999, Willis et al. 1999). This expectation is the implicit
basis for the myriad arguments favoring the existence of so-called “trade-offs” between
pathogenesis-affecting traits with other fitness traits in organisms composing
pathosystems. Our results support the hypothesis that hosts have relatively greater
evolutionary potential to mediate traits affecting the probability of infection, and that
pathogens have greater potential to mediate traits affecting the intensity of infection.
More detailed discussion of the patterns of adaptation in these traits follows.

4.4.3 Patterns of local adaptation and specialization in *Ipomoea*

and *Coleosporium ipomoeae*

4.4.3.1 Infectivity

Using mixed-model analysis to control for variation in our small sample of
locations, we found a significant trend of local adaptation of host populations in their
ability to resist infection from local vs. relatively distant pathogen races. In contrasts
designed to compare the infectivity of the pathogen races we studied, we found that
four pathogen races were locally maladapted in infectivity, evidenced by their lesser average probability of establishing infection on local vs. distant host populations. Five of the remaining six pathogen races showed a trend of local adaptation, though our contrasts did not find these races’ infectivities on local vs. distant hosts to be significantly different. One pathogen race, MO:P, showed slightly greater predicted infectivity on distant host populations, though the contrast of this difference was also not significant.

We found a more significant trend toward host specialization, and specialized resistance, in this pathosystem. With only one exception (CB:C), all pathogen races were better able to establish infection on their native host species than on non-native host species, and among these specialized races, contrasts comparing infectivity on the two kinds of host background were significant in all cases except that of CRG:P. Studies that find specialization in antagonistically coevolving systems are not uncommon, supporting the theoretical generalization that specialization is often favored in natural systems (Gould 1979, Agrawal 2000, Turner and Elena 2000, Little et al. 2006, Magalhaes et al. 2009).

That pathogen race CB:C was significantly better able to infect non-native host species is interesting. We expect local adaptation on average, but not always, in pathosystems due to frequency dependence in pathosystem coevolution (Gandon and
Van Zandt 1998), but there is not a complementary expectation for host specialization. If all hosts are equipotential to pathogens in that interactions are determined almost exclusively by the configuration of resistance specificities and pathogenicity factors, then it is reasonable to expect that host specialization and local adaptation would assume similar temporal dynamics due to frequency dependent effects. However, if host variation partitioned across species lines is important in the determination of host potential to pathogens, then we may not be able to explain by this reasoning a pathogen race whose performance is greater in its potential, but not realized, non-native host range in nature relative to its performance on its native host. In any case, a naturally-occurring pathogen race with greater infectivity in potential host backgrounds than on its realized host deserves further study. It is possible that it is an occurrence which is common, however short-lived, and representing the effects of spatiality of interactions: a recent and isolated migrant on a previously unrealized host resource.

4.4.3.2 Aggressiveness

Generally, pathogen races were locally adapted and specialized in aggressiveness, and host populations were neither locally adapted nor maladapted, but were specialized in their effects on aggressiveness. Our results describing differences in aggressiveness for sympatric vs. allopatric inoculations for individual host populations
were equivocal, with the exception of host population CB:C. That pathogen race CB:C
was exceptional in its pattern of infectivity, and host population CB:C is exceptional in
its pattern of aggressiveness, makes more attractive our hypothesis that the CB:C host-
pathogen interaction is unique among those we studied.

Six of ten contrasts of pathogen race aggressiveness on native vs. non-native host
species were significant. Eight of ten contrasts of pathogen race aggressiveness on home
host populations (i.e. the only category of host-pathogen interaction realized in nature)
vs. the average aggressiveness of races on all "away" populations were significant.
Pathogen races' local adaptation and specialization (and in this case, the significant
combination of these two traits) in aggressiveness is consistent with our hypothesis that
pathogen evolutionary potential to affect aggressiveness is greater than host potential to
affect aggressiveness, and should thus show general patterns of local adaptation.

One consideration which is important to an analysis of aggressiveness data is
that aggressiveness may be under balancing selection due to aggressiveness' possibly
experiencing frequency-dependent selection, or that aggressiveness may trade-off with
other traits which underlie pathogen fitness. If either is generally the case, the
aggressiveness of pathogen races in interactions with allopatric host populations may
show either higher or lower values, and there would be little reason to predict that any
given interaction would result in aggressiveness that is higher or lower than either that of the pathogen with its home population, or of any other given interaction (Dybdahl and Storfer 2003). In other words, if intermediate aggressiveness confers optimal pathogen race fitness on home hosts, then we expect optimal aggressiveness against away host populations to vary such that maximal aggressiveness is not fitness-maximizing optimal aggressiveness. However, such selection for a level of aggressiveness which confers optimal fitness for a pathogen race, and selection for increasing aggressiveness due to superinfection-mediated competition, would be in direct conflict. For two reasons, we expect that a history of selection for intermediate aggressiveness does not confound our analysis of the observed pattern of aggressiveness where we approximate greater fitness to greater aggressiveness: 1) maintenance of multiple pathogenesis-affecting alleles in pathogen populations may be expected because of the necessarily frequency-dependent nature of host-pathogen coevolutionary dynamics, as in the trench warfare model of coevolution (Stahl et al. 1999); 2) for communities of Ipomoea separated by only tens of kilometers, we expect pathogen metapopulation processes of migration and colonization to result in superinfection with adequate frequency to act antagonistically to hypothetical selection for intermediate aggressiveness.
4.4.3.3 Relationship between infectivity and aggressiveness

The theoretically expected relationship of infectivity to aggressiveness is a negative one. Though encumbered by a lack of standardized terms, literature characterizing relationships of the qualitative determination to the quantitative intensity of infection agrees that when a relationship exists, it exists in the form of a negative correlation or triangular relationship. Such a triangular relationship is identifiable by its reflecting all combinations of infectivity and aggressiveness values except for those where both infectivity and aggressiveness are high for one pathogen race. The interpretation of a triangular relationship in settings such as these has been, historically, that the factor preventing observation of highly infective and highly aggressive pathogen races in nature is a pleiotropic constraint, or a “trade-off” mediated by genetic linkage (Thrall et al. 2002, Sicard et al. 2007).

We observe such a triangular relationship in the average aggressiveness of each pathogen race to its average infectivity (Figure 13), and in the average aggressiveness of each pathogen race on its home host population to its average allopatric infectivity (Figure 14). Both of these relationships are reasonably expected to reflect the generally negative correlation of infectivity and aggressiveness predicted by theory, and observed in other empirical studies (Thrall and Burdon 2003, Sicard et al. 2007), because they relate...
realized aggressiveness which is under natural selection to potential host range, which affects pathogens’ ability to establish infection after migration events (Hellgren et al. 2009 demonstrate such a linkage between potential host range and prevalence in avian blood parasites).

However, we find no such relationship between pathogen races’ average allopatric aggressiveness and their allopatric infectivity, or between races’ average aggressiveness on their respective home host population and their infectivity on populations of non-native host species (Figure 15 and Figure 16). Here we do not necessarily expect a relationship to exist, because the comparison is between characteristics of a realized interaction and characteristics of one not realized in nature - potentially affected by correlations with traits under natural selection, but not under natural selection itself. The presence of such relationships in other pathosystems has been interpreted to represent “pre-adaptation” to the infection of novel hosts after migration (Parker and Gilbert 2004, Hellgren et al. 2009).

Finally, the relationship shown in Figure 17 is puzzling. Here we see a negative relationship between two traits characterizing interactions not realized in nature: average infectivity of pathogen races on non-native hosts, and average aggressiveness of pathogen races on non-native hosts. We hypothesize that though this relationship will
not necessarily bear on existing host-pathogen associations in nature, it will play an important role in determining the fates of novel host-pathogen associations which result from metapopulation processes such as propagule and allelic migration. Pathogen genotypes which confer both high infectivity and high aggressiveness are likely to successfully establish infection after pathogen migration events, because 1) the odds of a highly infective pathogen establishing infection on a random, novel host population are higher than those of a minimally infective pathogen establishing infection, and 2) a highly aggressive pathogen is likely to competitively exclude less aggressive pathogens from a given host background if rates of superinfection are adequately high. We are thus confronted with the question, if pre-adaptation to infect novel host populations confers potential fitness benefit to pathogens and is realized with adequate frequency, why should we not find pathogen races characterized by high values of both potential infectivity and potential aggressiveness? We hypothesize that the answer reflects the importance of host variation in determining the relationships of infectivity and aggressiveness in both realized and potential host-pathogen associations: highly aggressive pathogens impose strong selection for resistance on hosts. A highly aggressive and highly infective pathogen is likely to successfully infect and exert such selection on host populations for the same reasons as it is likely to be successful in
establishing infection after migration events. We thus expect pathogen genotypes conferring both high infectivity and high aggressiveness on the overall host metapopulation background to be excluded, by the evolution of resistance in host populations, at a greater rate than will be less aggressive pathogen genotypes. We suggest that this relationship is thus a reflection of both host evolution, and of past metapopulation process events.

4.4.3.4 Effects of multiple host species

In a pathosystem consisting of multiple host species, natural selection imposed on each host species by one generalist pathogen may be similar, but the genetic mechanisms of evolution in each host species are independent if there is no hybridization. Where host evolutionary potential to affect infectivity is greater than that of pathogens, and where host evolution is partitioned by species boundaries whereas pathogen evolution is not, host populations of one species within a multi-species community can evolve resistance independently, and we expect them to do so if pathogenesis-imposed selection favors resistance. The evolution of resistance in one host species in a multi-host community results in reduction of the respective pathogen’s host range, and increased intensity of selection to maintain infectivity on host species which are not resistant.
Our results show that host populations are locally adapted to resist infection from the pathogen races they commonly encounter in nature, but that pathogen races are host-specialized and better able to infect their native host species than they are able to infect hosts of other species, measured both in terms of pathogen races’ infectivity and aggressiveness. The degree to which pathogen races are host specialized is greater than the degree to which they are locally adapted to infect their home hosts, suggesting either that there is more host variation between species in traits affecting host-pathogen interactions (quantitative interactions especially) than there is variation between host communities, or that there are frequency-dependent dynamics occurring within individual host species independently of others (Kaltz and Shykoff 1998). The scale of coevolution in this pathosystem is reflected by these results, as well: that most host populations are locally adapted to resist infection from the pathogen races they encounter suggests that coevolution occurs at the scale of the location/community. Such small spatial scale of coevolutionary interaction between obligately biotrophic pathogens and their hosts has been found in similar plant-rust pathosystems (especially the natural Linum marginale – Melampsora lini pathosystem which has been well-studied in Australia by Thrall and Burdon, in which the spatial scale of divergence has been measured in hundreds of meters [Thrall et al. 2002]), and highlights the importance of
spatial scale and associated metapopulation processes in determining the evolutionary
dynamics of the pathosystem as a whole, or at least at relatively greater scope.

Our experiments provide evidence that local adaptation and specialization are
common in the *Ipomoea-Coleosporium* pathosystem. However, whether it is host
populations, pathogen races, or both which are locally adapted or specialized depends
on the trait studied. We have shown that hosts are locally adapted for infectivity, a trait
which affects the ability of pathogens to establish infection on given host plans, in all-or-
nothing fashion. Pathogens, on the other hand, are generally locally adapted, and
highly host-specialized, measured by a trait which affects the intensity of infection
resulting from a given compatible host-pathogen combination. The prediction that this
pattern would be observed derives from the fact that infectivity- and aggressiveness-
determining traits are controlled by different genetic mechanisms (Frank 2000, Hochberg
and Holt 2002), and that the evolutionary dynamics of the two traits are subject to
different influences (Rigby et al. 2002). We conclude that in this pathosystem, host and
pathogen local adaptation and specialization contribute both to the observed patterns of
infection, and to the fates of novel (and potential) host-pathogen associations which
result from migration.
5. Conclusion

The *Ipomoea-Coleosporium* pathosystem holds great potential for research into the effects of host species diversity on disease ecology and evolution. This work has highlighted the potential for such diversity to lead to genetic diversity while simultaneously resulting in consistent community structure across geographic distribution, and the potential for host species variation to contribute to the variable selection within the geographic mosaic of coevolution, as different host species each engage in arms races with a single pathogen species and thus diverge.

Future work in this natural pathosystem should include further study of pathogen genetic variation affecting interactions with hosts. For the conclusion that the interactions in this system adhere to gene-for-gene expectations requires complementary study in the pathogen, and will allow the interesting result of non-allelic variation for resistance in *I. hederacea* to be more substantially reconciled with our hypotheses concerning evolutionary potential: is it true that though host populations may vary in the locus at which resistance to one pathogen race is determined, only one pathogen locus is involved across all host populations?

Another important aspect of future work in this system should be a focus on the role of the pathogen’s complex life cycle in generating, or restricting, diversity. Because the pathogen undergoes sex and meiosis on its primary host, pines, there is the possibility that annual genetic recombination leads to generation of variation which is
selectively filtered on *Ipomoea* hosts each summer. If so, each summer’s associations could vary slightly – perhaps appreciably for aggressiveness but negligibly for infectivity – though we have little reason to think we can predict how this variation would look. Alternatively, pathogens races’ competing on hosts for the duration of summer, but then returning *en masse* to pines each year, could mean that expected adaptation toward intensity of host-pathogen associations is slowed by the fact that variation is “re-set” each year. In this case, pathogen genotypes’ returning to association with given hosts would require the compound contingency that 1) the pathogen genotypes have persisted through the annual population bottleneck of winter; 2) the genotypes are represented after recombination and mating may have eliminated them; 3) physical association of pathogen genotypes with host populations resumes due to the highly chance distribution of spores onto plants each spring; and, 4) plant populations persist both in presence and in susceptibility. The possibilities for the effects of heteroecy on this pathosystem are many, and with abundant variation having been documented here, these possibilities merit investigation.
Appendix A: Tables and Figures for Chapter 2

Figure 1: Approximate locations of host and pathogen collections used in genetic crosses

Table 1: Crosses to determine genetic basis of resistance, #1: *I. purpurea* species

<table>
<thead>
<tr>
<th>Source</th>
<th>No. R S2s</th>
<th>No. S S2s</th>
<th>Ratio R : S</th>
<th>LR χ2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2 x B1</td>
<td>41</td>
<td>13</td>
<td>3.15 : 1</td>
<td>0.0247</td>
</tr>
<tr>
<td>B4 x A2</td>
<td>39</td>
<td>14</td>
<td>2.79 : 1</td>
<td>0.0566</td>
</tr>
<tr>
<td>A5 x B7</td>
<td>46</td>
<td>17</td>
<td>2.71 : 1</td>
<td>0.1323</td>
</tr>
<tr>
<td>B8 x A8</td>
<td>42</td>
<td>16</td>
<td>2.63 : 1</td>
<td>0.2069</td>
</tr>
<tr>
<td>A9 x B9</td>
<td>51</td>
<td>17</td>
<td>3 : 1</td>
<td>0.0000</td>
</tr>
<tr>
<td>B12 x A11</td>
<td>40</td>
<td>14</td>
<td>2.86 : 1</td>
<td>0.0247</td>
</tr>
<tr>
<td><strong>Pooled</strong></td>
<td><strong>259</strong></td>
<td><strong>91</strong></td>
<td><strong>2.85 : 1</strong></td>
<td><strong>0.1867</strong></td>
</tr>
</tbody>
</table>

No LR χ2 values were significant
Table 2: Crosses to determine genetic basis of resistance, #2: *I. purpurea* species

**Cross 2: *I. purpurea* from locations CRG and LF**

Lines C: Host population CRG:P, susceptible to pathogen race CRG:P
Lines D: Host population LF:P resistant to pathogen race CRG:P
Fisher’s exact test for homogeneity, p=0.9641

<table>
<thead>
<tr>
<th>Source</th>
<th>No. R S2s</th>
<th>No. S S2s</th>
<th>Ratio R : S</th>
<th>LR χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 x D1</td>
<td>52</td>
<td>17</td>
<td>3.06 : 1</td>
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</tr>
<tr>
<td>D2 x C4</td>
<td>50</td>
<td>16</td>
<td>3.13 : 1</td>
<td>0.0202</td>
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<tr>
<td>C5 x D3</td>
<td>45</td>
<td>17</td>
<td>2.65 : 1</td>
<td>0.1935</td>
</tr>
<tr>
<td>D4 x C8</td>
<td>37</td>
<td>13</td>
<td>2.85 : 1</td>
<td>0.0267</td>
</tr>
<tr>
<td>C10 x D6</td>
<td>41</td>
<td>15</td>
<td>2.73 : 1</td>
<td>0.0952</td>
</tr>
<tr>
<td>D7 x C13</td>
<td>42</td>
<td>19</td>
<td>2.21 : 1</td>
<td>1.2295</td>
</tr>
<tr>
<td><strong>Pooled</strong></td>
<td><strong>267</strong></td>
<td><strong>97</strong></td>
<td>2.75 : 1</td>
<td><strong>0.5275</strong></td>
</tr>
</tbody>
</table>

*No LR χ² values were significant*

Table 3: Crosses to determine genetic basis of resistance, #3: *I. hederacea* species

**Cross 3: *I. hederacea* from locations CRG and LF**

Lines E: Host population CRG:H, susceptible to pathogen race LF:P
Lines G: Host population LF:H resistant to pathogen race LF:P
Fisher’s exact test for homogeneity, p=0.9244

<table>
<thead>
<tr>
<th>Source</th>
<th>No. R S2s</th>
<th>No. S S2s</th>
<th>Ratio R : S</th>
<th>LR χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>E5 x G3</td>
<td>68</td>
<td>21</td>
<td>3.24 : 1</td>
<td>0.0936</td>
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<tr>
<td>G10 x E9</td>
<td>64</td>
<td>25</td>
<td>2.56 : 1</td>
<td>0.4532</td>
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<tr>
<td>E14 x G11</td>
<td>69</td>
<td>23</td>
<td>3 : 1</td>
<td>0.0000</td>
</tr>
<tr>
<td>G14 x E16</td>
<td>60</td>
<td>21</td>
<td>2.86 : 1</td>
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<tr>
<td><strong>Pooled</strong></td>
<td><strong>261</strong></td>
<td><strong>90</strong></td>
<td>2.9 : 1</td>
<td><strong>0.0769</strong></td>
</tr>
</tbody>
</table>

*No LR χ² values were significant*
Table 4: Crosses to determine genetic basis of resistance, #4: *I. coccinea* species

**Cross 4: *I. coccinea* from locations CRG and MO**

Lines H: Host population CRG:C, susceptible to pathogen race MO:H
Lines J: Host population MO:C resistant to pathogen race MO:H
Fisher’s exact test for homogeneity, p=0.9641

<table>
<thead>
<tr>
<th>Source</th>
<th>No. R S2s</th>
<th>No. S S2s</th>
<th>Ratio R : S</th>
<th>LR $\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1 x J2</td>
<td>31</td>
<td>9</td>
<td>3.44 : 1</td>
<td>0.1333</td>
</tr>
<tr>
<td>J3 x H2</td>
<td>36</td>
<td>11</td>
<td>3.27 : 1</td>
<td>0.0638</td>
</tr>
<tr>
<td>H6 x J4</td>
<td>29</td>
<td>9</td>
<td>3.22 : 1</td>
<td>0.0351</td>
</tr>
<tr>
<td>J7 x H9</td>
<td>18</td>
<td>7</td>
<td>2.57 : 1</td>
<td>0.1200</td>
</tr>
<tr>
<td>H10 x J8</td>
<td>22</td>
<td>10</td>
<td>2.2 : 1</td>
<td>0.6667</td>
</tr>
<tr>
<td>J14 x H11</td>
<td>35</td>
<td>12</td>
<td>2.92 : 1</td>
<td>0.0071</td>
</tr>
<tr>
<td>H13 x J17</td>
<td>18</td>
<td>7</td>
<td>2.57 : 1</td>
<td>0.1200</td>
</tr>
<tr>
<td>J18 x H18</td>
<td>30</td>
<td>12</td>
<td>2.5 : 1</td>
<td>0.2857</td>
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<tr>
<td><strong>Pooled</strong></td>
<td><strong>219</strong></td>
<td><strong>77</strong></td>
<td><strong>2.84 : 1</strong></td>
<td><strong>0.1622</strong></td>
</tr>
</tbody>
</table>

No LR $\chi^2$ values were significant

Table 5: Crosses to test for allelism of resistance

**Tests of allelism**

<table>
<thead>
<tr>
<th>Source</th>
<th>No. R S2s</th>
<th>No. S S2s</th>
<th>Probability of false negative*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRG:H x LF:H, R to CRG:C</td>
<td>297</td>
<td>11</td>
<td>n/a</td>
</tr>
<tr>
<td>CL:P x LF:P, R to CRG:P</td>
<td>122</td>
<td>0</td>
<td>0.000381</td>
</tr>
<tr>
<td>CL:P x LF:P, R to Ellis:P</td>
<td>155</td>
<td>0</td>
<td>0.000045</td>
</tr>
</tbody>
</table>
Appendix B: Tables and Figures for Chapter 3

Table 6: Community locations, compositions, and infection status. I: host species present and infected by *C. ipomoeae*; P: host species present but uninfected; dash: host species absent.

<table>
<thead>
<tr>
<th>Location Name</th>
<th>Lat N</th>
<th>Lon W</th>
<th><em>I. coccinea</em></th>
<th><em>I. hederacea</em></th>
<th><em>I. purpurea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>36.01</td>
<td>78.92</td>
<td>P</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BA</td>
<td>35.21</td>
<td>79.25</td>
<td>-</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>BP</td>
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<td>79.26</td>
<td>-</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>BR</td>
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<td>I</td>
</tr>
<tr>
<td>CB</td>
<td>35.32</td>
<td>79.29</td>
<td>I</td>
<td>I</td>
<td>-</td>
</tr>
<tr>
<td>CC</td>
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<td>79.25</td>
<td>-</td>
<td>I</td>
<td>P</td>
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<td>79.26</td>
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<td>P</td>
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<td>P</td>
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<td>P</td>
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<td>I</td>
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<tr>
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<td>-</td>
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<td>I</td>
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<td>-</td>
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<td>41.91</td>
<td>87.67</td>
<td>-</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>
Figure 2: Composite matrix of compatibility reactions from experimental inoculation experiments over three years. Each host species is abbreviated to the first letter of its epithet. Cells occupied by the number 1 indicate combinations of rust isolate with plant populations which are compatible with frequency >0.7. Cells occupied by 0 indicate combinations with frequency <0.3. Cells occupied by the letter X indicated combinations in which between 30% and 70% of plants were compatible. The asterisked number 1 indicates a marginal compatibility result, between 60% and 70%. Empty cells are those for which experimental inoculations were not carried out.
Figure 3: Compatibility matrix condensed to highlight differences between rust isolates, and between host populations. Black brackets connect isolates or populations which are, based on the extent of our data, not able to be differentiated by their profiles of compatibility. Notable is that both pairs of similar genotypes are found across the CB and CL locations, which are the two locations nearest each other in our study, separated by 7.4 km.

Figure 4: Frequency of infection for four experimental inoculation categories
Figure 5: Most likely tree recovered by maximum likelihood search, RaxML, 1000 bootstrap replicates. Bold branches have >50% bootstrap support. Taxon names are [location]-[host species] [collection number].
Figure 6: Frequency of resistance after experimental inoculation.
Appendix C: Tables and Figures for Chapter 4

Figure 7: Approximate positions of locations within North Carolina chosen for inclusion in cross-inoculation experiments.

<table>
<thead>
<tr>
<th>Location Name</th>
<th><em>l. coccinea</em></th>
<th><em>l. hederacea</em></th>
<th><em>l. purpurea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>CB</td>
<td>I</td>
<td>I</td>
<td>-</td>
</tr>
<tr>
<td>CL</td>
<td>-</td>
<td>I</td>
<td>P</td>
</tr>
<tr>
<td>CRG</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>LF</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>MO</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
</tbody>
</table>

Figure 8: Infection status of studied host populations. “I” = host species present at location and infected with *C. ipomoeae*; “P” = host species present at location but not infected; “-” = host species absent from location.
Figure 9: Life cycle of *Coleosporium ipomoeae*.
Figure 10: Pathogen races’ average aggressiveness on native host species populations, in sympatric vs. allopatric inoculations.

Figure 11: Pathogen races’ average infectivity, on populations of native vs. non-native host species.
Figure 12: Pathogen races’ average aggressiveness, on home vs. the average on all away populations.
Figure 13: Relationship of average aggressiveness to average infectivity for each pathogen race.
Figure 14: Relationship of average aggressiveness on home host population to average allopatric infectivity for each pathogen race.
Figure 15: Relationship of average allopatric aggressiveness to average allopatric infectivity, for each pathogen race.
Figure 16: Relationship of average aggressiveness on home host population to average infectivity on non-native hosts, for each pathogen race.
Figure 17: Relationship of average aggressiveness on non-native hosts to average infectivity on non-native hosts.
Figure 18: Fit of Studentized residuals from mixed model analysis of aggressiveness to normal distribution.
Table 7: Planned contrasts of infectivity resulting from sympatric vs. allopatric inoculations, for individual pathogen races.

<table>
<thead>
<tr>
<th>Pathogen Race</th>
<th>Local Adaptation of Pathogen Infectivity</th>
<th>Inoculations of Greater Infectivity</th>
<th>Contrasts of Sympatric vs. Allopatric Inoculations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>df</td>
</tr>
<tr>
<td>CRG:C</td>
<td>Allopatric</td>
<td>1</td>
<td>10.42</td>
</tr>
<tr>
<td>LF:C</td>
<td>Allopatric</td>
<td>1</td>
<td>10.28</td>
</tr>
<tr>
<td>CB:H</td>
<td>Allopatric</td>
<td>1</td>
<td>19.93</td>
</tr>
<tr>
<td>CL:H</td>
<td>Allopatric</td>
<td>1</td>
<td>17.26</td>
</tr>
<tr>
<td>CRG:H</td>
<td>Allopatric</td>
<td>1</td>
<td>16.42</td>
</tr>
<tr>
<td>LF:H</td>
<td>Allopatric</td>
<td>1</td>
<td>13.81</td>
</tr>
<tr>
<td>MO:H</td>
<td>Allopatric</td>
<td>1</td>
<td>13.89</td>
</tr>
<tr>
<td>CRG:P</td>
<td>Allopatric</td>
<td>1</td>
<td>10.71</td>
</tr>
<tr>
<td>LF:P</td>
<td>Allopatric</td>
<td>1</td>
<td>9.415</td>
</tr>
<tr>
<td>MO:P</td>
<td>Sympatric</td>
<td>1</td>
<td>11.23</td>
</tr>
</tbody>
</table>

Significance after adjustment for familywise α: * p < 0.05, ** p < 0.005, *** p < 0.0005
Table 8: Planned contrasts of infectivity resulting from inoculations of native vs. non-native host species, for individual pathogen races.

<table>
<thead>
<tr>
<th>Pathogen Race:</th>
<th>Host Specialization of Pathogen Infectivity</th>
<th>Inoculations of Greater Infectivity</th>
<th>Contrasts of Inoculations on Native vs. Non-native Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ndf</td>
<td>ddf</td>
</tr>
<tr>
<td>CB:C</td>
<td>Non-native</td>
<td>1</td>
<td>11.43</td>
</tr>
<tr>
<td>CRG:C</td>
<td>Native</td>
<td>1</td>
<td>10.38</td>
</tr>
<tr>
<td>LF:C</td>
<td>Native</td>
<td>1</td>
<td>11.13</td>
</tr>
<tr>
<td>CB:H</td>
<td>Native</td>
<td>1</td>
<td>20.7</td>
</tr>
<tr>
<td>CL:H</td>
<td>Native</td>
<td>1</td>
<td>14.29</td>
</tr>
<tr>
<td>CRG:H</td>
<td>Native</td>
<td>1</td>
<td>17.78</td>
</tr>
<tr>
<td>LF:H</td>
<td>Native</td>
<td>1</td>
<td>13.64</td>
</tr>
<tr>
<td>MO:H</td>
<td>Native</td>
<td>1</td>
<td>15.11</td>
</tr>
<tr>
<td>CRG:P</td>
<td>Native</td>
<td>1</td>
<td>9.39</td>
</tr>
<tr>
<td>LF:P</td>
<td>Native</td>
<td>1</td>
<td>11.12</td>
</tr>
<tr>
<td>MO:P</td>
<td>Native</td>
<td>1</td>
<td>11.01</td>
</tr>
</tbody>
</table>

Significance after adjustment for familywise $\alpha$: * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$
Table 9: Planned contrasts of infectivity resulting from sympatric vs. allopatric inoculations, for individual host populations.

<table>
<thead>
<tr>
<th>Local Adaptation of Host Resistance</th>
<th>Inoculations of Greater Infectivity</th>
<th>Contrasts of Sympatric vs. Allopatric Inoculations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host Population:</td>
<td>ndf</td>
<td>ddf</td>
</tr>
<tr>
<td>CB:C</td>
<td>Allopatric 1</td>
<td>13.58</td>
</tr>
<tr>
<td>CRG:C</td>
<td>Allopatric 1</td>
<td>13.07</td>
</tr>
<tr>
<td>LF:C</td>
<td>Allopatric 1</td>
<td>13.49</td>
</tr>
<tr>
<td>MO:C</td>
<td>Allopatric 1</td>
<td>18.18</td>
</tr>
<tr>
<td>CB:H</td>
<td>Allopatric 1</td>
<td>19.19</td>
</tr>
<tr>
<td>CL:H</td>
<td>Allopatric 1</td>
<td>23.56</td>
</tr>
<tr>
<td>CRG:H</td>
<td>Allopatric 1</td>
<td>16.58</td>
</tr>
<tr>
<td>LF:H</td>
<td>Allopatric 1</td>
<td>14.28</td>
</tr>
<tr>
<td>MO:H</td>
<td>Allopatric 1</td>
<td>23.42</td>
</tr>
<tr>
<td>CRG:P</td>
<td>Allopatric 1</td>
<td>11.56</td>
</tr>
<tr>
<td>LF:P</td>
<td>Allopatric 1</td>
<td>12</td>
</tr>
<tr>
<td>MO:P</td>
<td>Sympatric 1</td>
<td>12.97</td>
</tr>
</tbody>
</table>

Significance after adjustment for familywise α: * p < 0.05, ** p < 0.005, *** p < 0.0005
Table 10: Planned contrasts of infectivity resulting from inoculations of native vs. non-native host species, for individual host populations.

<table>
<thead>
<tr>
<th>Specialization of Host Resistance</th>
<th>Inoculations of Greater Infectivity</th>
<th>Contrasts of Inoculations from Native vs. Non-native Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host Population:</td>
<td>ndf</td>
<td>ddf</td>
</tr>
<tr>
<td>CB:C</td>
<td>Native</td>
<td>1</td>
</tr>
<tr>
<td>CRG:C</td>
<td>Native</td>
<td>1</td>
</tr>
<tr>
<td>LF:C</td>
<td>Native</td>
<td>1</td>
</tr>
<tr>
<td>MO:C</td>
<td>Native</td>
<td>1</td>
</tr>
<tr>
<td>CB:H</td>
<td>Native</td>
<td>1</td>
</tr>
<tr>
<td>CL:H</td>
<td>Native</td>
<td>1</td>
</tr>
<tr>
<td>CRG:H</td>
<td>Native</td>
<td>1</td>
</tr>
<tr>
<td>LF:H</td>
<td>Native</td>
<td>1</td>
</tr>
<tr>
<td>MO:H</td>
<td>Native</td>
<td>1</td>
</tr>
<tr>
<td>CRG:P</td>
<td>Native</td>
<td>1</td>
</tr>
<tr>
<td>LF:P</td>
<td>Native</td>
<td>1</td>
</tr>
<tr>
<td>MO:P</td>
<td>Native</td>
<td>1</td>
</tr>
</tbody>
</table>

Significance after adjustment for familywise α: * p < 0.05, ** p < 0.005, *** p < 0.0005

Table 11: Covariance parameter estimates from mixed model analysis of aggressiveness.

<table>
<thead>
<tr>
<th>Covariance Parameter</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>Wald Z value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum source location nested within host species</td>
<td>0.010670</td>
<td>0.005414</td>
<td>1.97</td>
<td>0.0244</td>
</tr>
<tr>
<td>Inoculum target location nested within host species</td>
<td>0.003573</td>
<td>0.001708</td>
<td>2.09</td>
<td>0.0182</td>
</tr>
<tr>
<td>Residual</td>
<td>0.005021</td>
<td>0.000281</td>
<td>17.84</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Table 12: Planned contrasts of aggressiveness resulting from sympatric vs. allopatric inoculations, for individual pathogen races.

<table>
<thead>
<tr>
<th>Pathogen Race:</th>
<th>Local Adaptation of Pathogen Aggressiveness</th>
<th>Inoculations of Greater Aggressiveness</th>
<th>Contrasts of Sympatric vs. Allopatric Inoculations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ndf</td>
<td>Ddf</td>
</tr>
<tr>
<td>CRG:C</td>
<td>Sympatric</td>
<td>1</td>
<td>8.72</td>
</tr>
<tr>
<td>LF:C</td>
<td>Sympatric</td>
<td>1</td>
<td>8.7</td>
</tr>
<tr>
<td>CB:H</td>
<td>Sympatric</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td>CL:H</td>
<td>Sympatric</td>
<td>1</td>
<td>9.06</td>
</tr>
<tr>
<td>CRG: H</td>
<td>Sympatric</td>
<td>1</td>
<td>8.97</td>
</tr>
<tr>
<td>LF: H</td>
<td>Sympatric</td>
<td>1</td>
<td>8.92</td>
</tr>
<tr>
<td>MO: H</td>
<td>Sympatric</td>
<td>1</td>
<td>9.02</td>
</tr>
<tr>
<td>CRG: P</td>
<td>Sympatric</td>
<td>1</td>
<td>8.82</td>
</tr>
<tr>
<td>LF: P</td>
<td>Sympatric</td>
<td>1</td>
<td>8.66</td>
</tr>
<tr>
<td>MO: P</td>
<td>Sympatric</td>
<td>1</td>
<td>8.65</td>
</tr>
</tbody>
</table>

Significance after adjustment for familywise α: * p < 0.05, ** p < 0.005, *** p < 0.0005

Table 13: Planned contrasts of aggressiveness resulting from inoculations of native vs. non-native host species, for individual pathogen races.

<table>
<thead>
<tr>
<th>Pathogen Race:</th>
<th>Host Specialization of Pathogen Aggressiveness</th>
<th>Inoculations of Greater Aggressiveness</th>
<th>Contrasts of Inoculations on Native vs. Non-native Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ndf</td>
<td>Ddf</td>
</tr>
<tr>
<td>CRG:C</td>
<td>Native</td>
<td>1</td>
<td>8.54</td>
</tr>
<tr>
<td>LF:C</td>
<td>Native</td>
<td>1</td>
<td>8.43</td>
</tr>
<tr>
<td>CB:H</td>
<td>Native</td>
<td>1</td>
<td>10.3</td>
</tr>
<tr>
<td>CL:H</td>
<td>Native</td>
<td>1</td>
<td>9.17</td>
</tr>
<tr>
<td>CRG:H</td>
<td>Native</td>
<td>1</td>
<td>9.19</td>
</tr>
<tr>
<td>LF:H</td>
<td>Native</td>
<td>1</td>
<td>8.99</td>
</tr>
<tr>
<td>MO:H</td>
<td>Native</td>
<td>1</td>
<td>9.01</td>
</tr>
<tr>
<td>CRG: P</td>
<td>Native</td>
<td>1</td>
<td>8.38</td>
</tr>
<tr>
<td>LF: P</td>
<td>Native</td>
<td>1</td>
<td>8.83</td>
</tr>
<tr>
<td>MO: P</td>
<td>Native</td>
<td>1</td>
<td>8.82</td>
</tr>
</tbody>
</table>

Significance after adjustment for familywise α: * p < 0.05, ** p < 0.005, *** p < 0.0005
Table 14: Planned contrasts of aggressiveness resulting from inoculations of home/native hosts vs. others, for individual pathogen races.

<table>
<thead>
<tr>
<th>Population Specialization of Pathogen Aggressiveness</th>
<th>Inoculations of Greater Aggressiveness</th>
<th>Contrasts of Inoculations on Home/Native vs. Other Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population</td>
<td>df</td>
</tr>
<tr>
<td>CRG:C</td>
<td>Home population</td>
<td>1</td>
</tr>
<tr>
<td>LF:C</td>
<td>Home population</td>
<td>1</td>
</tr>
<tr>
<td>CB:H</td>
<td>Home population</td>
<td>1</td>
</tr>
<tr>
<td>CL:H</td>
<td>Home population</td>
<td>1</td>
</tr>
<tr>
<td>CRG:H</td>
<td>Home population</td>
<td>1</td>
</tr>
<tr>
<td>LF:H</td>
<td>Home population</td>
<td>1</td>
</tr>
<tr>
<td>MO:H</td>
<td>Home population</td>
<td>1</td>
</tr>
<tr>
<td>CRG:P</td>
<td>Home population</td>
<td>1</td>
</tr>
<tr>
<td>LF:P</td>
<td>Home population</td>
<td>1</td>
</tr>
<tr>
<td>MO:P</td>
<td>Home population</td>
<td>1</td>
</tr>
</tbody>
</table>

Significance after adjustment for familywise α: * p < 0.05, ** p < 0.005, *** p < 0.0005
Table 15: Planned contrasts of aggressiveness resulting from inoculations of home/native vs. allopatric/native hosts, for individual pathogen races.

<table>
<thead>
<tr>
<th>Pathogen Race</th>
<th>Local Adaptation of Pathogen Aggressiveness on Native Hosts</th>
<th>Inoculations of Greater Aggressiveness</th>
<th>Contrasts of Inoculations on Home/Native vs. Away/Native Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n df</td>
</tr>
<tr>
<td>CL:H</td>
<td>Home population</td>
<td></td>
<td>1 47</td>
</tr>
<tr>
<td>CRG:C</td>
<td>Home population</td>
<td></td>
<td>1 40 7.17*</td>
</tr>
<tr>
<td>CRG:H</td>
<td>Home population</td>
<td></td>
<td>1 43 42.83**</td>
</tr>
<tr>
<td>CRG:P</td>
<td>Home population</td>
<td></td>
<td>1 29 30.17*</td>
</tr>
<tr>
<td>LF:C</td>
<td>Home population</td>
<td></td>
<td>1 34 4.83</td>
</tr>
<tr>
<td>LF:H</td>
<td>Away populations</td>
<td></td>
<td>1 52 10.23</td>
</tr>
<tr>
<td>LF:P</td>
<td>Away populations</td>
<td></td>
<td>1 23.3 38.54*</td>
</tr>
<tr>
<td>MO:H</td>
<td>Home population</td>
<td></td>
<td>1 43 17.04*</td>
</tr>
<tr>
<td>MO:P</td>
<td>Home population</td>
<td></td>
<td>1 28 2.94</td>
</tr>
</tbody>
</table>

Significance after adjustment for familywise α: * p < 0.05, ** p < 0.005, *** p < 0.0005
Table 16: Planned contrasts of aggressiveness resulting from sympatric vs. allopatric inoculations, for individual host populations.

<table>
<thead>
<tr>
<th>Host Population:</th>
<th>Inoculations of Greater Aggressiveness</th>
<th>Contrasts of Sympatric vs. Allopatric Inoculations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Local Adaptation of Quantitative Host Resistance</td>
<td>ndf</td>
</tr>
<tr>
<td>CB:C</td>
<td>Sympatric</td>
<td>1</td>
</tr>
<tr>
<td>CRG:C</td>
<td>Sympatric</td>
<td>1</td>
</tr>
<tr>
<td>LF:C</td>
<td>Sympatric</td>
<td>1</td>
</tr>
<tr>
<td>MO:C</td>
<td>Sympatric</td>
<td>1</td>
</tr>
<tr>
<td>CB:H</td>
<td>Sympatric</td>
<td>1</td>
</tr>
<tr>
<td>CL:H</td>
<td>Sympatric</td>
<td>1</td>
</tr>
<tr>
<td>CRG:H</td>
<td>Sympatric</td>
<td>1</td>
</tr>
<tr>
<td>LF:H</td>
<td>Sympatric</td>
<td>1</td>
</tr>
<tr>
<td>MO:H</td>
<td>Sympatric</td>
<td>1</td>
</tr>
<tr>
<td>CRG:P</td>
<td>Sympatric</td>
<td>1</td>
</tr>
<tr>
<td>LF:P</td>
<td>Sympatric</td>
<td>1</td>
</tr>
<tr>
<td>MO:P</td>
<td>Sympatric</td>
<td>1</td>
</tr>
</tbody>
</table>

Significance after adjustment for familywise α: * p < 0.05, ** p < 0.005, *** p < 0.0005
Table 17: Planned contrasts of aggressiveness resulting from inoculations of native vs. non-native host species, for individual host populations.

<table>
<thead>
<tr>
<th>Specialization of Quantitative Host Resistance</th>
<th>Inoculations of Greater Aggressiveness</th>
<th>Contrasts of Inoculations from Native vs. Non-native Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host Population:</td>
<td>ndf</td>
<td>df</td>
</tr>
<tr>
<td>CB:C</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>CRG:C</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>LF:C</td>
<td>1</td>
<td>15.2</td>
</tr>
<tr>
<td>MO:C</td>
<td>1</td>
<td>15.4</td>
</tr>
<tr>
<td>CB:H</td>
<td>1</td>
<td>13.1</td>
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<tr>
<td>CL:H</td>
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<td>13.2</td>
</tr>
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<td>CRG:H</td>
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<td>12.6</td>
</tr>
<tr>
<td>LF:H</td>
<td>1</td>
<td>14.3</td>
</tr>
<tr>
<td>MO:H</td>
<td>1</td>
<td>12.9</td>
</tr>
<tr>
<td>CRG:P</td>
<td>1</td>
<td>14.8</td>
</tr>
<tr>
<td>LF:P</td>
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<td>13.4</td>
</tr>
<tr>
<td>MO:P</td>
<td>1</td>
<td>14.5</td>
</tr>
</tbody>
</table>

Significance after adjustment for familywise α: * p < 0.05, ** p < 0.005, *** p < 0.0005
References


Kniskern JM, Rausher MD. 2006b. Major-gene resistance to the rust pathogen
Coleosporium ipomoeae is common in natural populations of Ipomoea purpurea. New
Phytologist, 171: 137–144.

Kniskern JM, Rausher MD. 2007. Natural selection on a polymorphic disease-resistance

Lajeunesse MJ, Forbes MR. 2002. Host range and local parasite adaptation. Proceedings of
the Royal Society of London, Series B 269(1492): 703-710.


the basis of parasite adaptation. Evolution 60(1): 31-38.


Maddison DR, Maddison WP. 2001. MacClade 4: Analysis of Phylogeny and Character

Magalhães S, Blanchet E, Egas M, Olivieri I. 2009. Are adaptation costs necessary to
build up a local adaptation pattern? BMC Evolutionary Biology 9:182.

Gazette 131(2): 139-144.

for polymorphism in the disease resistance gene Rps2 of Arabidopsis thaliana.
Genetics 163: 735-746.

Meyers BC, China DB, Shena KA, Sivaramakrishnana S, Lavellea DO, Zhanga Z,
Michelmore RW. 1998. The major resistance gene cluster in lettuce is highly


Michelmore RW, Meyers BC. 1998. Clusters of Resistance Genes in Plants Evolve by


Biography

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University of Michigan, Ann Arbor  B.S. magna cum laude, Biology  2004
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National Science Foundation Doctoral Dissertation Improvement Grant, 2009

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