

Major-gene resistance to the rust pathogen *Coleosporium ipomoeae* is common in natural populations of *Ipomoea purpurea*

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Summary

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- The genetic basis of resistance to pathogens is well studied in crops, yet our understanding of the evolution of this trait in natural populations will be improved by determining how resistance is inherited in a wide range of plant–pathogen interactions. Here, we examined resistance to *Coleosporium ipomoeae*, a common fungal rust pathogen of *Ipomoea purpurea*.
- Natural populations across North Carolina, South Carolina, and Georgia (USA) were surveyed for the presence of *C. ipomoeae* and seeds were collected. A combination of crosses and controlled infections was then used to determine the genetic basis of qualitative resistance.
- In one population studied in detail, complete resistance to natural infection and a bulk collection of *C. ipomoeae* is conferred by a single locus (*Rci1*), where resistance is dominant to susceptibility. Allelic, major-gene resistance to this same bulk collection of *C. ipomoeae* appears to also occur in nine other natural populations.
- The prevalence of this resistance phenotype in natural populations suggests that the evolution of resistance to *C. ipomoeae* in *I. purpurea* may be dominated by genes of large phenotypic effect.

Key words: major-gene resistance, plant–pathogen interaction, coevolution, *Coleosporium*, *Ipomoea*.

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Introduction

In agricultural systems, one of the primary mechanisms of plant resistance to pathogens involves gene-for-gene interactions (Crute & Pink, 1996; Agrios, 1997). Such an interaction is characterized by allelic variation at a single locus in the host and at a single locus in the pathogen, such that infection success is determined by the combined genotypes of host and pathogen. Typically, a dominant allele confers resistance in the host and a recessive allele confers virulence in the pathogen. The host is resistant only if it carries at least one copy of the resistance allele and the pathogen carries one copy of the avirulence allele (Flor, 1971). Over the past decade,

molecular dissection of plant–pathogen interactions exhibiting this type of resistance has revealed that, in most instances, the host-resistance locus (R-gene) codes for a protein that recognizes the product of the pathogen avirulence gene directly, or recognizes the interaction of the avirulence gene product and its target (Ellis *et al.*, 2000; Dangl & Jones, 2001). Recognition initiates a cascade of physiological changes in the plant that ultimately results in the hypersensitive response and systemic acquired resistance.

Deployment of novel R-genes in crops generally confers short-lived control of disease, because the new resistance gene selects for virulence in the pathogen (Agrios, 1997). Repeated introductions of new R-genes, often at different loci, cause the

pathogen repeatedly to evolve virulence, generating a 'coevolutionary' sequence (e.g. wheat stem rust, Vanderplank, 1984; barley powdery mildew, Brown, 1984). This sequence of events has been adopted as a model of coevolution between plants and their pathogens in natural communities (Thompson & Burdon, 1992; Burdon, 1997; Holub, 2001), probably because it is relatively common in agricultural systems; the genetics of gene-for-gene interactions are appealingly simple; and many wild plants exhibit the kind of race-specific resistance that would be expected from interactions between R-genes and avirulence genes (Thompson & Burdon, 1992).

An alternative model of natural coevolution involves resistance that acts as a quantitative trait. In this model, level of resistance is governed by allelic composition at several to many loci, and may or may not be independent of the genotype of the pathogen (Geiger & Heun, 1989; Young, 1996). Such resistance is also known to plant breeders, who commonly term it polygenic or quantitative resistance, in contrast to gene-for-gene resistance, which is classified as monogenic or major-gene resistance (Agrios, 1997).

Currently there is little understanding of which of these models, if either, best characterizes variation for resistance to pathogens in the majority of natural plant populations (Thompson, 1994). Major-gene resistance to agriculturally important pests is common in relatives of crop species (Malcolmson & Black, 1966; Dinooor, 1977; Manisterksi *et al.*, 1986), and examples of major-gene resistance have also been found in wild plant-pathogen associations (Burdon, 1987; Harry & Clarke, 1987; Parker, 1988; Jarosz & Burdon, 1990; Bevan *et al.*, 1993; Burdon, 1994), although in many cases only one or a few populations were investigated. Other studies have documented genetic variation for quantitative resistance in natural populations (de Nooij & van Damme, 1988; Simms, 1993; Ericson *et al.*, 2002; Price *et al.*, 2004), but nothing is known about the actual number of loci involved. It is thus clear that a better understanding of the evolution (and coevolution) of plant resistance in nature will require further genetic characterization of resistance in natural plant populations.

In this report, we describe the genetics of variation for resistance in natural populations of the common morning glory, *Ipomoea purpurea*, to a common pathogen, *Coleosporium ipomoeae*. We demonstrate that, in one population, variation in susceptibility to *C. ipomoeae* collected from a single population is governed by allelic variation at a single locus. We also show that individuals vary in susceptibility to infection by *C. ipomoeae* in many populations throughout the south-eastern USA, and that segregation of this variation is consistent with control of resistance by the same major gene in different populations. Finally, we argue that the resistance characterized here is unlikely to be selectively neutral, and that divergence in the frequency of this phenotype among populations may reflect variation in the kinds of selective forces acting on major-gene resistance in nature.

Materials and Methods

Plants and pathogen

Ipomoea purpurea (L.) Roth (Convolvulaceae) is a weedy annual that is common in agricultural fields and other disturbed sites throughout south-eastern North America. Although *I. purpurea* is self-compatible, it predominantly outcrosses in nature with a frequency estimated to range from 65 to 74% (Ennos, 1981; Schoen & Clegg, 1985). Experimental plants used in crosses were obtained from our collection of inbred lines (Fineblum & Rausher, 1995; Tiffin & Rausher, 1999) that were originally sampled from a field in Durham County, NC, USA in 1989 (population PF). All lines used had been inbred for at least 12 generations of selfing and single-seed descent. Plants were also collected from 11 sites in North Carolina, South Carolina and Georgia. Locations of these sites are listed in Table 1. All plants were grown in fertilized potting soil (14-14-14) in a glasshouse or growth chamber (16-h days at 21°C constant temperature) in trays containing 21–36 pots, and were watered as necessary.

Coleosporium ipomoeae is a macrocyclic, heteroecious rust pathogen that occurs in the USA, Mexico, the West Indies, Central America and South America (Rhoads *et al.*, 1918). *Coleosporium ipomoeae* is known to use *Pinus* as its primary host, from which aeciospores are transmitted to alternative hosts of *Argyreaia*, *Calyptegia*, *Convolvulus*, *Jacquemontia* and *Ipomoea*, all in the family Convolvulaceae (Farr *et al.*, 1989). In macrocyclic, heteroecious rusts uredia (= uredinia) are formed on the alternate host and produce urediospores (= urediniospores) that are able to re-infect alternate hosts. Eventually, telia are formed on the alternate host and produce teliospores, from which basidiospores emerge to re-infect the primary host (Littlefield, 1981).

Controlled infection

Coleosporium ipomoeae urediospores were collected in bulk from a single population (TF) in North Carolina from many infected leaves of *I. purpurea* and *Ipomoea hederacea* Jacq. Consequently the number of different *C. ipomoeae* strains in this bulk collection is unknown. Unless explicitly stated otherwise, all the experiments reported here were conducted in a growth chamber with this single bulk collection of *C. ipomoeae*. To infect plants experimentally with *C. ipomoeae* the following procedure was used. Approximately 8 h before inoculation, plants were placed within a clear plastic bag to increase humidity. Inoculation solutions were prepared by adding dry urediospores to a 0.1% Tween 20 solution in a 1% weight-to-volume ratio. This solution was sprayed as a fine mist onto the underside of true leaves immediately before the dark cycle and plastic bags were removed approx. 48 h after inoculation. Infection was then allowed to progress naturally. Following controlled inoculations, susceptible

Table 1 Presence of *Coleosporium ipomoeae* in natural mixed *Ipomoea* populations in North Carolina (NC), South Carolina (SC) and Georgia (GA), USA, and frequency of line M resistance phenotype in *Ipomoea purpurea*

State	Population	Latitude*	Longitude*	<i>I. purpurea</i>	<i>I. hederacea</i>	<i>I. lacunosa</i>	<i>I. coccinea</i>	Frequency of resistance† (SE)
NC	CS	36 06	80 15	P	P	N	N	0.11 (0.06)
NC	BP	36 06	79 26	P	P	P	–	‡
NC	TF	36 06	79 26	P	P	–	–	‡
NC	CF	36 06	79 26	N	P	–	N	‡
NC	MO	36 06	79 16	N	P	P	N	‡
NC	MC	35 49	80 15	–	P	N	–	‡
NC	TP	35 44	78 51	P	P	N	N	‡
NC	BR	35 44	78 51	P	P	N	N	‡
NC	FH	35 44	78 51	P	P	P	N	‡
NC	GT	35 42	79 49	P	P	P	N	‡
NC	C3	35 29	79 11	P	P	N	N	‡
NC	PR	35 29	79 11	P	P	–	–	0.3 (0.11)
NC	BA	35 21	79 25	P	P	–	–	‡
NC	PM	35 21	79 25	P	P	–	–	‡
NC	CC	35 21	79 25	N	P	–	–	‡
NC	CT	35 21	79 25	P	P	–	–	‡
NC	NP	35 21	79 25	P	P	P	–	‡
SC	OK	34 42	79 53	P	–	–	P	0.08 (0.06)
SC	IK	34 42	79 53	P	–	–	P	0.26 (0.09)
SC	CG	34 00	81 02	P	N	N	–	0.58 (0.15)
SC	PL	33 53	80 42	N	N	–	N	0.5 (0.11)
SC	CP	33 53	80 42	–	P	P	–	‡
SC	PC	33 48	80 38	N	P	–	–	1 (0)
SC	GS	33 48	80 38	P	N	P	–	0.61 (0.09)
GA	MO	32 41	83 21	P	P	–	–	0.57 (0.1)
GA	CF	32 36	82 20	P	N	–	–	0.45 (0.09)
GA	HG	32 27	83 44	P	P	–	–	0 (0)
GA	LT	32 05	83 48	P	P	P	–	‡

P, species present and infected with *C. ipomoeae*.

N, species present but not infected by *C. ipomoeae*.

–, Species not observed at this location.

*Approximate latitude and longitude in degrees and minutes.

†Frequency of resistance to bulk collection of *C. ipomoeae* exhibited by line M.

‡Frequency of resistance not assessed.

plants exhibit bright orange uredia on the underside of leaves after 10–14 d, while resistant plants show a complete absence of uredia and exhibit small, black flecks or larger black spots on the underside of leaves after 7–10 d, a phenotype that is referred to as the hypersensitive response (HR) and is commonly associated with resistance to rust pathogens (Heath, 1976). The bulk collection of *C. ipomoeae* was periodically propagated on susceptible plants to maintain adequate amounts of urediospores for these experiments.

Genetic characterization of resistance

Preliminary assays indicated that the inbred line M was consistently resistant to infection using the bulk collection of *C. ipomoeae*, while four lines (AA, C, D and L) were consistently susceptible. In one set of crosses, we crossed line M to each of three susceptible lines: C, D and L. One F_1 plant from each cross was selfed to generate F_2 seeds, which were

germinated and scored for susceptibility to infection. Infection of F_2 plants from the crosses with D and L were conducted in the growth chamber with the bulk collection of *C. ipomoeae*. The F_2 plants from the cross with line C were planted in early summer in a field in Durham County, NC in which *C. ipomoeae* was abundant, and were allowed to be infected naturally. Plants were scored for infection in the autumn (data from R. Zufall, with permission).

In a second experiment, F_2 plants from crosses with lines AA, D and L that had been identified as resistant or susceptible were allowed to self. Up to 12 F_3 plants from each resistant F_2 parent were then tested for resistance phenotype. Three susceptible plants from line C were included within each tray to serve as positive controls. The F_3 progeny of susceptible F_2 plants were not tested, but were assumed to be homozygous for susceptibility. Crosses were then made within and between resistant and susceptible F_3 plants, and six F_4 progeny were tested from each cross for both the presence of uredia and the type of HR (black flecks vs black spots).

Population surveys

Twenty-eight populations from North Carolina, South Carolina and Georgia (Table 1) were surveyed for the presence of four species of *Ipomoea* (*I. purpurea*, *I. hederacea*, *I. lacunosa* L. and *I. coccinea* L.), all of which have been reported as hosts of *C. ipomoeae* (Farr *et al.*, 1989). These surveys were made in the latter half of the growing season, when plants were flowering and setting mature seeds. Infection of each species was determined by thoroughly examining each plant in a population for *C. ipomoeae* uredia until at least one infected individual was found, or until an exhaustive search of all plants had been made. A species was thus considered infected if at least one individual was infected, but in most cases, when there was infection, many plants were infected. The frequency of plants that exhibited the same resistance phenotype as line M (strong HR and no uredia in response to the bulk collection of *C. ipomoeae*) was then estimated for 11 of these populations in growth-chamber infections. We sampled seeds from an average of 23 haphazardly chosen plants from each population (range: nine to 34). A single seed from each plant was grown and scored for resistance.

Genetics of resistance in survey populations

Soon after plants from natural populations were challenged with the bulk collection of *C. ipomoeae*, it became clear that most populations contain plants that are entirely susceptible (abundant uredia; no HR) and plants that exhibit the line-M phenotype (abundant HR; no uredia). To understand if this shared phenotype has a shared genetic basis, plants from the survey populations with the resistant phenotype were transferred to the glasshouse and allowed to self. If complete resistance to the bulk collection of *C. ipomoeae* in these populations is conferred by a single locus where resistance is dominant to susceptibility, then selfed (S_1) progeny of resistant plants should yield the same discrete phenotypes observed in progeny of line M plants (complete resistance or susceptibility). Furthermore, some proportion of resistant plants should be heterozygous, and these plants should yield the expected 3 : 1 ratio of resistant : susceptible phenotypes in the S_1 generation. To test this prediction, between 14 and 18 S_1 progeny from each resistant plant were generally scored for resistance phenotype, although poor germination yielded only 10–14 plants in a few cases.

Allelism tests

Allelism tests were conducted to evaluate the hypothesis that resistance alleles from natural *I. purpurea* populations throughout the south-eastern USA reside at different loci from the resistance allele in the PF population. For these tests, plants inferred to be homozygous for resistance from the population surveys (see above) were crossed to line M, a single

F_1 plant was selfed, and the resistance phenotypes of 87–96 F_2 progeny were determined. In addition, two plants (TF61 and TF81) identified as putatively homozygous for resistance from a different set of crosses, described elsewhere (Kniskern, 2004), were included in these allelism tests. All these plants were tested in groups of three to four trays of plants, and two plants from susceptible line C were included within each tray as positive controls.

Results

Inheritance of resistance within the PF population

Under controlled infection in the growth chamber using the bulk collection of *C. ipomoeae*, the ratio of resistant to susceptible F_2 progeny did not differ significantly, in either of two crosses, from the 3 : 1 ratio expected for segregation of a dominant resistance allele and a recessive susceptible allele at a single locus ($M \times D$: 51R and 17S (3.00 : 1), $\chi^2 = 0$, $P > 0.99$; $M \times L$: 41R and 16S (2.56 : 1), $\chi^2 = 0.2865$, $P > 0.5$). Similarly, under natural infection in the field the ratio of uninfected (presumed resistant) to infected (presumed susceptible) plants did not differ significantly from a 3 : 1 ratio ($M \times C$: 83R vs 28S (2.96 : 1), $\chi^2 = 0.003$, $P > 0.95$). When the results from all three crosses are pooled, there remains no significant deviation from expectation (175R and 61S (2.87 : 1), $\chi^2 = 0.09$, $P > 0.9$).

To verify single-locus inheritance of resistance to *C. ipomoeae*, we performed a set of supplementary crosses in which we first identified the presumed single-locus genotype of F_2 individuals derived from crosses involving the resistant line M and the susceptible lines AA, D and L. F_2 individuals were selfed, and up to 12 F_3 progeny of resistant F_2 plants were scored for resistance. All positive controls exhibited uredia as expected. Among the 19 resistant F_2 plants tested, 14 produced both resistant and susceptible progeny. Pooled over these 14 sets of progeny, 82 were resistant and 35 susceptible, a ratio that again does not differ from the 3 : 1 ratio expected for control by a single dominant locus (ratio = 2.34 : 1, $\chi^2 = 1.148$, $P > 0.1$).

The remaining five resistant F_2 plants produced no susceptible F_3 offspring, and were thus presumed to be homozygous for resistance (RR). Selfed F_3 progeny from these resistant F_2 plants, along with 12 F_3 progeny of susceptible F_2 plants (presumed homozygous susceptible, rr), were then crossed among themselves in various combinations. A total of 15 crosses were made to generate homozygous resistant F_4 progeny (RR \times RR); heterozygous progeny (RR \times rr); and homozygous susceptible progeny (rr \times rr). Six F_4 progeny from each cross were scored for resistance, and each exhibited the resistance phenotype expected based on the inferred genotypes of the parents. Specifically, all offspring predicted to be resistant consistently lacked *C. ipomoeae* uredia and exhibited the HR, while plants predicted to be susceptible consistently supported uredia growth and lacked the HR.

Table 2 Resistance phenotypes of F_1 progeny from resistant plants collected from survey populations and selfed

State	Population	F_1 families without segregation (R only)	F_1 families with segregation (R&S)	Resistant F_1 * (from segregated families)	Susceptible F_1 † (from segregated families)	Ratio R : S
NC	CS	0	3	39	9	4.33 : 1
NC	PR	2	4	53	8	6.63 : 1
SC	CG	3	4	56	14	4 : 1
SC	GS	12	8	78	24	3.25 : 1
SC	IK	3	3	38	8	4.75 : 1
SC	OK	2	0	–	–	–
SC	PC	8	1	12	3	4 : 1
SC	PL	6	5	74	15	4.93 : 1
GA	CF	7	6	76	32	2.38 : 1
GA	MO	9	7	90	33	2.73 : 1
	SUM	52	41	516	146	3.53 : 1

*Number of resistant F_1 plants from segregating F_1 families pooled within populations.

†Number of susceptible F_1 plants from segregating F_1 families pooled within populations.

Table 3 Goodness-of-fit tests for proportion of resistant to susceptible plants from segregating F_1 families

Tests	State _{population} NC _{CS}		NC _{PR}		SG _{CG}		SC _{GS}		SC _{IK}		SC _{PC}		SC _{PL}		GA _{CF}		GA _{MO}	
	df	G	df	G	df	G	df	G	df	G	df	G	df	G	df	G	df	G
Pooled†	1	1.1	1	5.3*	1	1.0	1	0.1	1	1.5	1	0.2	1	3.4	1	1.2	1	0.2
Heterogeneity‡	2	0.8	3	0.8	3	5.2	7	10.9	2	1.0	0	–	4	0.8	5	7.4	6	2.3
Total	3	1.9	4	6.1	4	6.2	8	11.0	3	2.5	1	0.2	5	4.2	6	8.6	7	2.5

*, G-test significant at $P < 0.05$.

†Tests the hypothesis that pooled ratio deviates from expected 3 : 1 ratio of a single gene where resistance is dominant to susceptibility.

‡Tests the hypothesis that the progeny in segregating F_1 families are significantly heterogeneous within a population.

We had previously observed two distinct HR phenotypes, one (HR-A) characterized by small, dark flecks on the underside of leaves; the other (HR-B) characterized by larger, grey spots on the underside of leaves, with some plants exhibiting both types. The proportion of plants exhibiting these two types did not differ between plants that were inferred to be homozygous resistant and those inferred to be heterozygotes according to a G -test of independence ($G = 0.02$, $P > 0.75$). Consequently, the morphology of the hypersensitive response does not appear to be influenced by genotype at the presumed resistance locus.

Population surveys

Our population surveys indicate that some populations of *I. purpurea* are uninfected by *C. ipomoeae* (Table 1). In most cases, the rust infected other *Ipomoea* species growing at the same site, although at one site (PL) the rust was absent from all species.

Controlled infections of plants from some of the survey populations revealed that the frequency of plants that exhibit the line-M (resistant) phenotype varies substantially among populations (Table 1). While most populations had both resistant and susceptible phenotypes, only resistant plants

were identified from one population, and only susceptible plants from another. A heterogeneity G -test based on the eight populations exhibiting both phenotypes shows these populations to be significantly different in phenotypic frequency ($G_H = 38.75$, $df = 8$, $P < 0.001$). These results indicate that the phenotype originally observed in line M (complete resistance to the bulk collection of *C. ipomoeae*) is quite common in natural populations.

Genetics of resistance in survey populations

Plants expressing the line-M phenotype of complete resistance to the bulk collection of *C. ipomoeae* were recovered from 10 of the 11 populations sampled. These plants were allowed to self, and nine of the 10 populations examined had at least one resistant plant that yielded susceptible selfed offspring; in most populations many resistant plants produced susceptible progeny (Table 2).

Within populations, there was no evidence for heterogeneity among tested plants in the ratio of resistant to susceptible offspring (Table 2). Only one population (PR) exhibited a nominally significant departure from a 3 : 1 resistant : susceptible segregation ratio (Table 3, $P = 0.02$), and this significance disappears when a Bonferroni correction is made for

Table 4 Summary of allelism tests

Parents	F_2	Resistant	Susceptible	Probability of results under H_A^*
TF 61 × M	88	88	0	0.00342
TF 81 × M	87	87	0	0.00364
PR 1 × M	88	88	0	0.00342
PR 15 × M	96	96	0	0.00204
IK 2 × M	93	93	0	0.00247
IK 3 × M	91	91	0	0.00281
OK 5 × M	96	96	0	0.00204
OK 8 × M	92	92	0	0.00264

*Probability of obtaining results under the alternative hypothesis that resistance in other populations is conferred by a locus unlinked to the *Rci1* locus present in the resistant inbred line M.

multiple comparisons. Moreover, a heterogeneity G -test reveals no significant difference among populations in the segregation ratio ($G_H = 10.87$, $df = 8$, $P > 0.1$). This lack of significance justifies pooling results across populations, which yields no significant departure from a 3 : 1 segregation ratio (pooled ratio 3.5 : 1, $\chi^2 = 3.06$, $df = 1$, $P > 0.075$). These results are consistent with the interpretation that variation in resistance to our bulk sample is determined in these populations, as in population PF, by segregation at a single locus with two alleles.

Allelism tests

As an initial attempt to determine whether resistance in the survey populations is controlled by the same locus as in the PF population, we crossed presumed homozygous resistant plants from each of four populations to the resistant M line from the PF population, and scored the resistance and HR phenotypes of the selfed F_2 progeny. If resistance in these populations is conferred by a resistance allele at a different, unlinked locus, then *c.* 1/16 of the progeny should be susceptible. In contrast, none of the 731 progeny tested from these populations was susceptible (Table 4). These results are consistent with control of resistance residing at the same locus in all populations examined, though with these data we cannot rule out the possibility that different, tightly linked loci are involved.

Discussion

Genetic control of resistance

In population PF, variation in resistance of *I. purpurea* to natural infection and infection by a bulk collection of *C. ipomoeae* appears to be controlled by variation at a single locus, which we designate *Rci1*. At this locus, the allele conferring resistance is completely dominant. In growth-chamber infections using a bulk collection of *C. ipomoeae*,

resistant genotypes exhibit a typical hypersensitive response that blocks infection completely: uredia are never found on resistant plants. Plants of the susceptible genotype, in contrast, lack a hypersensitive response; infection can result in hundreds of uredia per leaf. These observations suggest that the *Rci1* locus is involved in mediating the hypersensitive response. However, our data do not indicate whether *Rci1* codes for a receptor protein of the type characteristically involved in gene-for-gene interactions (Ellis *et al.*, 2000), or for a downstream gene in the HR pathway. Furthermore, we do not know how many strains of *C. ipomoeae* are present in our bulk collection, or when infection occurs naturally in the field. As a consequence, we cannot say whether *Rci1* provides resistance that is broad or narrow in spectrum.

Our surveys revealed that many populations throughout the south-eastern USA are variable in the frequency of plants that exhibit complete resistance to the bulk collection of *C. ipomoeae*, referred to here as the line-M phenotype. Although the work reported here represents only an initial characterization of the genetic basis of variation for resistance in these populations, our results are consistent with the interpretation that a major gene controlling resistance to our bulk samples is segregating in many *I. purpurea* populations throughout the south-eastern USA.

The results of our allelism tests are consistent with the hypothesis that the same major locus controls variation in resistance in the different populations examined. However, we cannot rule out the possibility that resistance alleles at different, tightly linked loci confer resistance, in different populations, to the same strain(s) of *C. ipomoeae* used here. This possibility deserves serious consideration because, in most plant species that have been examined, R-genes occur as multiple, tightly linked copies (Meyers *et al.*, 1999; Young, 2000). Furthermore, because these allelism tests were conducted with *C. ipomoeae* from a single population, it is possible that other populations harbor alleles at other R-gene loci that confer resistance to other genotypes of *C. ipomoeae*, especially if one or more complete cycles of coevolution have occurred between *I. purpurea* and *C. ipomoeae* independently in different populations (Kniskern & Rausher, 2001). Results from other systems indicate that the existence of multiple resistance loci may be common in natural plant populations (Burdon, 1987; Harry & Clarke, 1987; Bevan *et al.*, 1993; Burdon, 1994).

Evolution of resistance

We have found that, in our survey populations, segregation of resistance to our bulk *C. ipomoeae* sample is consistent with variation at a single locus with two alleles. In addition, our results are consistent with the hypothesis that the resistance factors from different populations are allelic. To the extent that these inferences are correct, these two results, in turn, suggest that the differences among populations in the

frequencies of plants resistant or susceptible to our bulk *C. ipomoeae* collection are caused by an underlying divergence in allele frequencies at *Rci1*. (We note that populations may also differ in other resistance factors not assayed by our bulk collection.) Although we currently lack any direct information on the causes of this divergence, it is unlikely to be caused entirely by genetic drift. *Coleosporium ipomoeae* is a potent pathogen of *I. purpurea* that substantially reduces plant fitness (Kniskern & Rausher, 2006). Moreover, resistant genotypes exhibit a substantial cost of resistance that can reach 15.5%; this cost generates a fitness differential at the *Rci1* locus at low pathogen densities (Kniskern, 2004). It thus seems unlikely that variation at this locus is neutral, allowing for divergence by drift.

Instead, at least two classes of explanation involving selection can be proposed to account for this variation. On one hand, differences in the frequency of resistance among populations may reflect differences in equilibrium gene frequencies at *Rci1*. Models of resistance evolution in single populations indicate that a cost of resistance, coupled with an inverse relationship between pathogen density and frequency of resistance, can yield a stable resistance polymorphism in which the equilibrium allele frequencies are determined by the magnitude of the cost and by the overall prevalence of the disease (Mode, 1958; Jayakar, 1970; Clarke, 1976; Leonard, 1977; Frank, 1992; Antonovics & Thrall, 1994). As both the magnitude of resistance costs (Bergelson, 1994) and disease abundance (Burdon *et al.*, 1989) are often influenced by environmental factors that can vary among locations, one would naturally expect geographical variation in the equilibrium frequency of resistance. On the other hand, frequency differences among populations may reflect the asynchronies among populations in nonequilibrium coevolutionary dynamics that are expected in metapopulations (Damgaard, 1999; Carlsson-Granér & Thrall, 2002; Thrall & Burdon, 2002). The characterization of the *Rci1* locus described here should facilitate distinguishing between these explanations.

Variation in disease prevalence

Our population surveys revealed that *C. ipomoeae* was absent from five of 26 *I. purpurea* populations examined. Examination of frequencies of resistant phenotypes, as well as whether the rust is present on other, co-occurring species of *Ipomoea* (Table 1), suggests that rust prevalence in *I. purpurea* populations is influenced by both genetic and environmental factors. For example, absence of rust in one population (PC) is probably caused primarily by genetic resistance. The presence of infected *I. hederacea* plants at the same site indicates that lack of infection in *I. purpurea* is not likely to be caused by absence of the rust at that site, as *I. hederacea* and *I. purpurea* germinate at similar times and are thus similarly exposed to windblown spores. Moreover, every plant tested from this population exhibited resistance to our bulk sample

of *C. ipomoeae*. In contrast, infection of *I. purpurea* was observed in every one of the seven populations in which some plants tested were susceptible to our bulk sample, and in which rust was known to be present because other *Ipomoea* species were infected. Based on this reasoning, we predict that at three other North Carolina sites (CF, MO and CC) where infection is absent in *I. purpurea* but present in congeners, most *I. purpurea* plants would be resistant. This prediction is supported by previous work showing that plant resistance may reduce pathogen prevalence among populations (Thrall & Burdon, 2000; Laine, 2004).

Absence of infection in *I. purpurea* at the PL site, in contrast, may be caused by the absence of *C. ipomoeae* at the site. The absence of infection in any of the three *Ipomoea* species at this site is consistent with this hypothesis, as is our observation that half the *I. purpurea* plants in this population are susceptible to our bulk rust sample. Natural plant populations often escape infection, and the likelihood of this occurring is related to the size and proximity of an inoculum source and the mode of pathogen dispersal (reviewed by Burdon *et al.*, 1989). Nevertheless, we cannot rule out the possibility that, at the PL site, all three species are completely resistant to the local strains of *C. ipomoeae*. Although these conclusions are tentative, these intriguing patterns suggest that the interaction between *Ipomoea* and *C. ipomoeae* constitutes a good system for exploring the evolutionary dynamics of plants and their pathogens.

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References

- Agrios GN. 1997. *Plant Pathology*. London, UK: Academic Press.
- Antonovics J, Thrall PH. 1994. The cost of resistance and the maintenance of genetic polymorphism in host–pathogen systems. *Proceedings of the Royal Society of London B* 257: 105–110.
- Bergelson J. 1994. The effects of genotype and the environment on costs of resistance in lettuce. *American Naturalist* 143: 349–359.
- Bevan JR, Clarke DD, Crute IR. 1993. Resistance to *Erysiphe fischeri* in two populations of *Senecio vulgaris*. *Plant Pathology* 42: 636–646.
- Brown JKM. 1984. Chance and selection in the evolution of barley mildew. *Trends in Microbiology* 2: 470–475.
- Burdon JJ. 1987. Phenotypic and genetic patterns of resistance to the pathogen *Phakopsora pachyrhizi* in populations of *Glycine canescens*. *Oecologia* 73: 257–267.
- Burdon JJ. 1994. The distribution and origin of genes for race-specific resistance to *Melampsora lini* in *Linum marginale*. *Evolution* 48: 1564–1575.

- Burdon JJ. 1997. The evolution of gene-for-gene interactions in natural pathosystems. In: Crute IR, Holub EB, Burdon JJ, eds. *The Gene-for-Gene Relationship in Plant-Parasite Interactions*. Oxford, UK: CAB International.
- Burdon JJ, Jarosz AM, Kirby GC. 1989. Pattern and patchiness in plant-pathogen interactions – causes and consequences. *Annual Review of Ecology and Systematics* 20: 119–136.
- Carlsson-Granér U, Thrall PH. 2002. The spatial distribution of plant populations, disease dynamics and evolution of resistance. *Oikos* 97: 97–110.
- Clarke B. 1976. The ecological genetics of host-parasite relationships. In: Taylor ER, Muller R, eds. *Genetic Aspects of Host-Parasite Relationships*. Oxford, UK: Blackwell Scientific.
- Crute IR, Pink DAC. 1996. Genetics and utilization of pathogen resistance in plants. *Plant Cell* 8: 1747–1755.
- Damgaard C. 1999. Coevolution of a plant host-pathogen gene-for-gene system in a metapopulation model without cost of resistance or cost of virulence. *Journal of Theoretical Biology* 201: 1–12.
- Dangl JL, Jones JDG. 2001. Plant pathogens and integrated defence responses to infection. *Nature* 411: 826–833.
- Dinoor A. 1977. Oat crown rust resistance in Israel. *Annals of the New York Academy of Sciences* 287: 357–366.
- Ellis J, Dodds P, Pryor T. 2000. Structure, function and evolution of plant disease resistance genes. *Current Opinion in Plant Biology* 3: 278–284.
- Ennos RA. 1981. Quantitative studies of the mating system in two sympatric species of *Ipomoea* (Convolvulaceae). *Genetica* 57: 93–98.
- Ericson L, Burdon JJ, Müller WJ. 2002. The rust pathogen *Triphragmium ulmariae* as a selective force affecting its host, *Filipendula ulmaria*. *Journal of Ecology* 90: 167–178.
- Farr DF, Bills GF, Chamuris GP, Rossman AY. 1989. *Fungi on Plants and Plant Products in the United States*. St Paul, MN, USA: APS Press.
- Fineblum WL, Rausher MD. 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. *Nature* 377: 517–520.
- Flor HH. 1971. The current status of gene-for-gene concept. *Annual Review of Phytopathology* 9: 275–296.
- Frank SA. 1992. Models of plant-pathogen coevolution. *Trends in Genetics* 8: 213–219.
- Geiger HH, Heun M. 1989. Genetics of quantitative resistance to fungal diseases. *Annual Review of Phytopathology* 27: 317–341.
- Harry IB, Clarke DD. 1987. The genetics of race-specific resistance in groundsel (*Senecio vulgaris*) to the powdery mildew *Erysiphe fischeri*. *New Phytologist* 107: 715–723.
- Heath M. 1976. Hypersensitivity, the cause or consequence of rust resistance? *Phytopathology* 67: 935–936.
- Holub EB. 2001. The arms race is ancient history in Arabidopsis, the wildflower. *Nature Reviews. Genetics* 2: 516–527.
- Jarosz AM, Burdon JJ. 1990. Predominance of a single major gene for resistance to *Phakopsora pachyrhizi* in a population of *Glycine argyrea*. *Heredity* 64: 347–353.
- Jayakar SD. 1970. A mathematical model for interaction of gene frequencies in a parasite and its host. *Theoretical Population Biology* 1: 140–164.
- Kniskern JM. 2004. Natural selection on a disease-resistance gene in *Ipomoea purpurea*. PhD thesis, Duke University, Durham, NC, USA.
- Kniskern JM, Rausher MD. 2001. Two modes of host-enemy coevolution. *Population Ecology* 43: 3–14.
- Kniskern JM, Rausher MD. 2006. Environmental variation mediates the deleterious effects of *Coleosporium ipomoeae* on its host, *Ipomoea purpurea*. *Ecology* 87: 675–685.
- Laine A. 2004. Resistance variation within and among host populations in a plant-pathogen metapopulation: implications for regional pathogen dynamics. *Journal of Ecology* 92: 990–1000.
- Leonard KJ. 1977. Selection pressures and plant pathogens. *Annals of the New York Academy of Sciences* 287: 207–222.
- Littlefield LJ. 1981. *Biology of the Plant Rusts*. Ames, IA, USA: Iowa State University Press.
- Malcolmson JF, Black W. 1966. New R genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. *Euphytica* 15: 199–203.
- Manisterksi J, Treeful L, Tomerlin JR, Anikster Y, Moseman JG, Wahl I, Wilcoxson RD. 1986. Resistance of wild barley accessions from Israel to leaf rust collected in the USA and Israel. *Crop Science* 21: 222–232.
- Meyers BC, Dickerman AW, Michelmore RW, Sivaramakrishnan S, Sobral BW, Young ND. 1999. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. *Plant Journal* 20: 317–332.
- Mode CJ. 1958. A mathematical model for the co-evolution of obligate parasites and their hosts. *Evolution* 12: 158–165.
- de Nooij MP, van Damme JMM. 1988. Variation in host susceptibility among and within populations of *Plantago lanceolata* L. infected by the fungus *Phomopsis subordinaria* (Desm.) Trav. *Oecologia* 75: 535–538.
- Parker MA. 1988. Polymorphism for disease resistance in the annual legume *Amphicarpaea bracteata*. *Heredity* 60: 27–31.
- Price JS, Bever JD, Clay K. 2004. Genotype, environment, and genotype by environment interactions determine quantitative resistance to leaf rust (*Coleosporium asterum*) in *Euthamia graminifolia* (Asteraceae). *New Phytologist* 162: 729–743.
- Rhoads AS, Hedgecock GG, Bethel E, Hartley C. 1918. Host relationships of the North American rusts, other than gymnosporangiums, which attack conifers. *Phytopathology* 7: 309–352.
- Schoen DJ, Clegg MT. 1985. The influence of flower color on outcrossing rate and male reproductive success in *Ipomoea purpurea*. *Evolution* 39: 1242–1249.
- Simms EL. 1993. Genetic variation for pathogen resistance in tall morning glory. *Plant Disease* 77: 901–904.
- Thompson JN. 1994. *The Coevolutionary Process*. Chicago, IL, USA: University of Chicago Press.
- Thompson JN, Burdon JJ. 1992. Gene-for-gene coevolution between plants and parasites. *Nature* 360: 121–125.
- Thrall PH, Burdon JJ. 2000. Effect of resistance variation in a natural plant host-pathogen metapopulation on disease dynamics. *Plant Pathology* 49: 767–773.
- Thrall PH, Burdon JJ. 2002. Evolution of gene-for-gene systems in metapopulations: the effect of spatial scale of host and pathogen dispersal. *Plant Pathology* 51: 169–184.
- Tiffin P, Rausher MD. 1999. Genetic constraints and selection acting on tolerance to herbivory in the common morning glory *Ipomoea purpurea*. *American Naturalist* 154: 700–716.
- Vanderplank JE. 1984. *Disease Resistance in Plants*. Orlando, FL, USA: Academic Press.
- Young ND. 1996. QTL mapping and quantitative disease resistance in plants. *Annual Review of Phytopathology* 34: 479–501.
- Young ND. 2000. The genetic architecture of resistance. *Current Opinion in Plant Biology* 3: 285–290.