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SPECIALIZATION AND LOCAL ADAPTATION OF A FUNGAL PARASITE ON TWO HOST PLANT SPECIES AS REVEALED BY TWO FITNESS TRAITS

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We investigate the geographic pattern of adaptation of a fungal parasite, *Colletotrichum lindemuthianum*, on two host species, *Phaseolus vulgaris* and *P. coccineus* for two parasite fitness traits: infectivity (ability to attack a host individual) and aggressivity (degree of sporulation and leaf surface damage). Using a cross-inoculation experiment, we show specialization of the fungus on its host species of origin for both traits even when fungi, which originated from hosts growing in sympatry, were tested on sympatric host populations. Within the two host species, we compared infectivity and aggressivity on local versus allopatric plant–fungus combinations. We found evidence for local adaptation for the two traits on *P. vulgaris* but not on *P. coccineus.* There was no significant correlation between the degrees of local adaptation for infectivity and aggressivity, indicating that the genetic basis and the effect of selection may differ between these two traits. For the two fitness traits, a positive correlation between the degree of specialization and the degree of local adaptation was found, suggesting that specialization can be reinforced by local adaptation.

KEY WORDS: **Aggressivity, coevolution, *Colletotrichum lindemuthianum,* infectivity, *Phaseolus*, virulence.**

No parasite exploits all potential host species. Many, if not most, parasites are further restricted in their host range to particular host types, populations, and even individuals. Two different levels of host range limitations by parasites have received much attention: specialization on particular species (Futuyma and Moreno 1988; Joshi and Thompson 1995) and local adaptation on particular populations (see for reviews Kaltz and Shykoff 1998; Lajeunesse and Forbes 2002; Kawecki and Ebert 2004). Both patterns may be the consequence of adaptation to particular host genotypes. They may arise if there are trade-offs in performance on

different host species or populations (Rausher 1995) or even in the absence of such trade-offs but when selection pressures are heterogeneous across hosts (Fry 1996; Kawecki 1998). Do these two levels of adaptation reflect the same underlying processes or may they be independent?

Local adaptation, that is, enhanced performance on sympatric host populations (Morand et al. 1996) can result from the process of rapidly evolving parasites adapting to the most common genotypes encountered within their populations (Hamilton et al. 1990). Local adaptation will arise when there are trade-offs between performances on different host genotypes and when gene flow is restricted in both host and parasite so that their populations are differentiated for resistance- and infection-related traits (Gandon et al. 1996; Gandon and Michalakis 2002).

Specialization evolves when host *species* differ in their aptitude as hosts (e.g., resistance, physiology, life history) and parasites more commonly encounter one species. This specialization may be qualitative, implying the inability to attack species other than the principal host species, that is, nonhosts (Delgado et al. 2001; Newcombe 2003; Wyand and Brown 2003; Atienza et al. 2004) or quantitative, with average poorer performance on nonhabitual hosts (e.g., Via 1991; Agrawal 2000). Specialization may have a fundamentally different genetic and mechanistic basis than that of within-species local adaptation (Mellersh and Heath 2003). Pathogen-associated-molecular-pattern (PAMP) -triggered immunity (PTI) renders plant species nonhosts to most potential parasites by responding to widespread pathogen proteins. This is different from race-specific resistance that relies on recognition of specific pathogen effector proteins. On the other hand, these pathogen effector proteins may also be implicated in blocking PTI. If effectors that block PTI, thereby turning a nonhost to a host, include the same avirulence factors that trigger race-specific induced resistance (Chisholm et al. 2006), there is a link between specific and general defense systems. Thus, where a common set of genes is involved (Elliott et al. 2002; Trujillo et al. 2004; Nurnberger and Lipka 2005), specialization may arise as a direct outcome of short-term selection for local performance (Thompson 1994).

Here we are interested in the relationship between local adaptation and specialization in host–parasite systems with closely related host species. We present the range of possibilities for this relationship in Figure 1. Some parasites (see Fig. 1A) may be total generalists both within and among host species. Some parasites may have a narrow host range but perform similarly across all populations of their host species (see Fig. 1B). Clearly, if performance declines monotonically with increasing genetic distance between hosts, then parasite populations will be both locally adapted and specialized (see Fig. 1D), and this may reflect the accepted notion that specialists are derived from generalists (Bernays and Graham

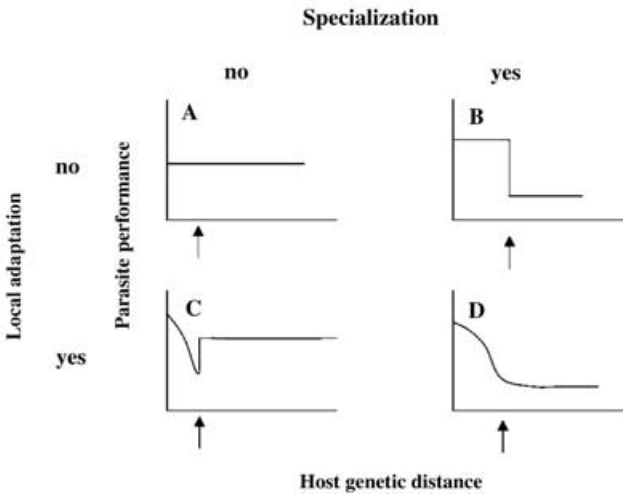

Figure 1. **Parasite performance is depicted as a function of the genetic distance between hosts, measured for characters relevant to host defense against parasites. The arrow represents the barrier between species. We present the four possible combinations of presence and absence of specialization and local adaptation. Cases A and B depict, respectively, generalist and specialist parasites that are not locally adapted. Case C presents a generalist parasite that is locally adapted while the parasite in Case D is both specialized and locally adapted.**

1988; Thompson 1994). The fourth case (see Fig. 1C) is more problematic because it is difficult to imagine genetic or selective mechanisms that could generate a parasite that is locally adapted but not specialized. This may be possible when the genetic basis for the two processes are independent, but this case may be unlikely for closely related hosts.

Which of these scenarios will arise in a natural coevolving system will depend on several factors. The genetic basis of adaptation to host and parasite respectively (e.g., Berenbaum and Zangerl 1998) and its genetic architecture will determine direct and correlated responses to selection (e.g., Fry 1996). Local processes will be modified by larger-scale effects in a metapopulation where there is gene flow among local demes each with its own selective history (Gandon et al. 1996) or even gene flow between closely related species. Furthermore, interacting species may differ somewhat in geographic range so the strength of local selection will vary across the range of a species (Thompson 1994) and patterns of local adaptation may vary among species with contrasting ranges (Lajeunesse and Forbes 2002).

Few studies have examined the relationship between local adaptation and specialization on different host species. Although quantitative specialization has been reported for many parasites (Via 1991; MacKenzie 1996; Traxler and Joern 1999; Agrawal 2000; Fry 2003a; Brandt and Foitzik 2004), investigations of multiple host and parasite populations, providing information about

local adaptation at the same time, are rare. Nonetheless, some studies have revealed variation in specialization among populations and/or parasite fitness components for a number of different herbivores (Via 1991; Thomas and Singer 1998; Traxler and Joern 1999; Ballabeni et al. 2003), showing that there can be a geographic component to specialization. Furthermore, while local adaptation has been found for numerous host–parasite interactions (see for reviews Kaltz and Shykoff 1998; Lajeunesse and Forbes 2002; Kawecki and Ebert 2004), few studies to date have considered local adaptation for multiple fitness components (but see Davelos et al. 1996; Mackinnon and Read 1999; Koskela et al. 2000; Kaltz and Shykoff 2002). Different scenarios may arise for different parasite fitness components, each having its own evolutionary trajectory, though genetic covariance between characters may render them nonindependent. For example, parasites are strongly selected to be as infectious as possible, but increased damage to hosts may decrease the longevity of an infection, ultimately reducing parasite fitness. Therefore, depending on the host population density, locally adapted parasites should infect a maximum of local hosts but not necessarily cause greatest damage (Kirchner and Roy 2002; Dybdahl and Storfer 2003). In this study, we investigate the relationship between quantitative specialization and local adaptation in a plant–pathogen system. We measure two parasite fitness components, infectivity (ability to attack a host individual) and aggressivity (fungal sporulation and degree of leaf surface damage). Aggressivity includes aspects of parasite reproduction and host damage that may or may not lead to a decrease in host fitness. Although parasite-induced host fitness loss is called "virulence" in the parasitological literature, we avoid this term both because the relationship between leaf damage and host fitness is complex (e.g., Jarosz and Davelos 1995; Roy and Kirchner 2000) and because, in the plant pathology literature, virulence has a completely different meaning, denoting the ability to infect a host individual, here called infectivity. Please also note that although we consider infectivity and aggressivity parasite fitness traits, both are, in many cases, determined by the interaction between host and parasite genotypes and should not be considered properties of either parasite or host exclusively (Salvaudon et al. 2005; Lambrechts et al. 2006). The gene-for-gene relationship of Flor (1956) is an excellent example of infectivity determined by the interaction between host and parasite genotypes (Thompson and Burdon 1992). Because the phenotype of a host–parasite interaction is determined by the two parties, traits measured on one may reflect the state of the other. For example, degree of sporulation, which we consider a parasite fitness component, is also a useful measure of host resistance (e.g., Edwards and Williams 1987).

Using a cross-inoculation experiment, we tested for the existence of host specialization and local adaptation in *Colletotrichum lindemuthianum,* a foliar fungal pathogen of the bean species *Phaseolus vulgaris* and *P. coccineus*. We further investigated the relationship between the degree of local adaptation and of specialization for these two parasite fitness traits.

Materials and Methods

STUDY ORGANISMS AND POPULATIONS

The parasite, *C. lindemuthianum* (Sacc. and Magnus) Lams.-Scrib., is a filamentous fungus responsible for bean anthracnose. Spore morphology and sequence data indicate that it belongs to the deuteromycota (Bailey et al. 1992). This fungus attacks leaves, pods, and flower tissue; it decreases seed germination and seedling death rates (Pastor-Corrales and Tu 1989). The fungus can complete its asexual life cycle both on living and nonliving hosts. Horizontal transmission results from rain splash, thus the spores move only a short distance (Tu 1992). Vertical transmission occurs through infected seeds. The fungus can be conserved from one host generation to the next on plant debris (Dillard and Cobb 1993). The sexual form of the fungus has never been detected in nature (Bryson et al. 1992). Fungal strains isolated from a range of natural populations are differentiated for neutral markers and virulence factors at local, regional, and country scales (Sicard et al. 1997a,b). Furthermore, fungal strains were locally adapted to their *P. vulgaris* host plants from their countries of origin (Geffroy et al. 1999). Local adaptation at the scale of individual host plant was also found (Capelle and Neema 2005). However, local adaptation of this fungus among populations within regions has never been investigated.

The two host plants are the common bean, *P. vulgaris*, and the runner bean, *P. coccineus*. Both species originated in Latin America. Natural populations of *P. vulgaris* are found from Mexico to North Argentina from 500 m to 2000 m elevation (Delgado-Salinas 1988; Gepts and Debouck 1991; Gepts 1998). Natural populations of *P. coccineus* are found in Central America from 1000 m to 3000 m elevation (Delgado-Salinas 1988). The two species are closely related and can interbreed. They differ, however, in several life history traits: *P. vulgaris* is predominantly self-pollinating whereas *P. coccineus* is predominantly cross-pollinating (Delgado-Salinas 1988; Escalante et al. 1994; Delgado-Salinas et al. 1999). Furthermore, *P. vulgaris* is an annual plant and usually occurs in small, isolated populations of fewer than 25 individuals that are often subject to local extinctions. *P. coccineus*, on the other hand, is a perennial plant and occurs in large, stable populations. Populations of *P. vulgaris* show significant genetic differentiation for neutral markers and resistance factors to *C. lindemuthianum* (Cattan-Toupance et al. 1998; De Meaux et al. 2003; Papa and Gepts 2003). In contrast, populations of *P. coccineus* show no significant genetic differentiation for neutral markers (Escalante et al. 1994). Whether *P. coccineus* populations show genetic differentiation for resistance factors to *C. lindemuthianum* is unknown.

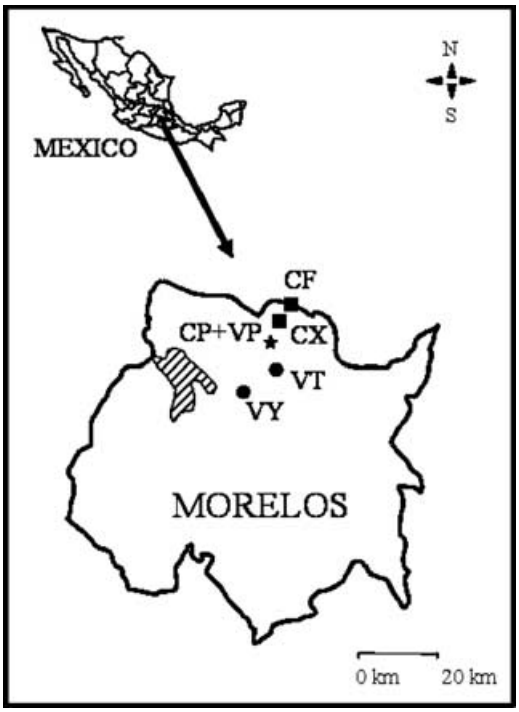

Figure 2. Geographical locations of the collected host–parasite populations. Circles indicate populations of *Phaseolus vulgaris* with no *P. coccineus* plants (VT and VY), squares indicate populations of *P. coccineus* with no *P. vulgaris* plants (CX and CF), and the star indicates the sympatric population of the two bean species (VP and CP).

Three wild *P. vulgaris* populations (named VP, VY, VT) and three wild populations of *P. coccineus* populations (CP, CF, CX) from the northern area of the state of Morelos, Mexico, were used for this study (see Fig. 2). Each population has a two-letter code, the first letter for host species (V for *P. vulgaris* and C for *P. coccineus*) and the second letter for the site (Y, T, P, F, or X). The populations were found on roadsides, on the borders of fields, and in dried riverbeds. They were distributed from the town of Yautepec close to the northern state border along an altitudinal gradient. Populations of *P. vulgaris* were located in the southeast, at altitudes ranging from 1264 m to 1965 m, and populations of *P. coccineus* were in the northwest at altitudes ranging from 1965 m to 2203 m. We also included one locality at an intermediate altitude (1965 m) in which there were sympatric populations of both host species. At this site, individual plants of the two species were mixed and leaves, flowers, and pods of the two species could be found only centimeters apart. Anthracnose was found in all locations. However, the incidence of the disease ranged from few to all plants infected depending on the location. Overall, there was more anthracnose damage on *P. vulgaris* than on *P. coccineus*. Sporulation was apparent on *P. vulgaris* leaves but never seen on *P. coccineus*.

COLLECTION AND TREATMENT OF SEEDS AND FUNGAL MATERIAL

Seeds and infected material were collected in December 1999. From each of the six populations, 10 seeds of 30 different mother plants were harvested. Offspring from a single mother plant will be referred to as a family.

Also from each population, infected material was harvested from different plants. Isolates of the fungus were obtained from infected pods and leaves as described in Sicard et al. (1997a). In total, 19 single spore isolates that will be referred to as fungal strains were obtained. Five strains were obtained from each of the three *P. vulgaris* populations, VP, VY, and VT. Three strains were obtained from the *P. coccineus* population CF and one strain from the *P. coccineus* population CP. No strains were isolated from the *P. coccineus* population CX. Isolation of the fungus from infected material of *P. coccineus* was less successful, probably because of the lack of sporulation on leaf surfaces. A set of strains obtained from the same host population will be referred to as a fungal population.

Seed dormancy was broken by scarification. Scarified seeds were first sown in moist vermiculite and germination type (hypogeous or epigeous) was recorded to ensure that the plant was of the right species. After two weeks, seedlings were planted in the greenhouse. Planting position was randomly assigned for individuals within each population and species, with population and species alternating along 20 rows placed 50 cm apart.

EXPERIMENTAL DESIGN AND PATHOGENICITY TEST

Infectivity and aggressivity were tested in a detached leaf assay that Geffroy et al. (2000) developed for this plant–parasite system to map quantitative resistance loci. Some of these quantitative resistance loci colocalized with specific resistance genes detected with whole plant assay, suggesting that the fungus infection on detached leaves triggers common plant defense response. Similarly, when detached leaf and whole plant assay were used to map quantitative resistance to *Phytophthora infestans* in tomato, several quantitative trait loci (QTLs) were consistently detected across assays (Brouwer et al. 2004). Indeed, detached leaf assays are commonly used in plant pathology to analyze plant defense mechanisms. These assays do not measure all components of resistance as expressed in natural populations. However, quantitative resistance can be difficult to assess because environmental, developmental, and physiological factors can influence the observed phenotypes. By increasing environmental control, such a test has been shown to increase heritability of plant resistance (Brouwer et al. 2004).

To control for developmental factors, the same trifoliate leaf was used to test fungal strains from different host populations. On a testing day, one trifoliate leaf was collected from each plant to be tested. Each of the three leaflets from the same trifoliate

leave was inoculated with a different fungal strain. For *P. vulgaris* plants, the first leaflet was inoculated with a *P. coccineus* strain, the second with a sympatric *P. vulgaris* strain, and the third with a randomly chosen allopatric *P. vulgaris* strain. A similar procedure was followed for *P. coccineus*, but where no sympatric strain was available, two leaflets were inoculated with strains from two different *P. vulgaris* populations.

Fresh inoculum made from 7- to 10-day-old sporulating culture and standardized to a concentration of 10^6 spores/ml was put on the leaflets using a paintbrush (Geffroy et al. 2000). Each inoculated leaflet was placed in a petri dish lined with moist filter paper. Leaflets were randomly distributed over the room and left for seven days in moist conditions at controlled temperature, which cycled from 19°C to 24°C over 24 h, with 24 h light. Symptoms were then recorded as follows. For infectivity, leaflets were either infected (1) or not (0), the latter showing no visible symptoms or only limited necrotic spots. For aggressivity, infected leaflets were scored on a three-point scale: 1, leaflet with lesions; 2, leaflet with sporulating and/or macerated large lesions; 3, sporulating completely macerated dead leaflets. Aggressivity is measured as the level of host damage, because macerated leaves fall and are lost to the host plant, but also reflects the possibility of spore transmission, which is facilitated when leaves become macerated.

Because it was impossible to carry out all inoculations at once, five groups of 360 plants each were inoculated at each of five inoculation dates between 9 June 2000 and 22 December 2000. Host species, populations, and plant families were equally represented across the five groups of plants. At each inoculation date, the order of leaf collection and leaflet inoculation were random and one randomly chosen strain from each population of *P. vulgaris* was used. The single strain from population CP of *P. coccineus* was inoculated at all dates, while the other strains from population CF of *P. coccineus* were inoculated at one of the inoculation dates. Therefore, strains of the same fungal population were tested at different dates and, thus, variability between strains of the same plant population also reflects the variability between inoculation dates.

REPEATABILITY OF THE EXPERIMENTAL DESIGN

We tested the repeatability of our inoculation procedure by repeating certain experiments. We used one-way analysis of variance (ANOVA) to calculate intraclass correlations on the symptom scores (Zar 1984). First, to test for consistency of inoculation and scoring, two people performed identical inoculations on the same day with one of three strains (VT4, VY2, VP3) on 157 leaflets collected from a random set of plants. The same two persons also scored the symptoms independently. Symptom scores for the same inoculated leaflet were consistent between independent scorers ($r_i = 0.863$, $F_{(313,314)} = 13.55$, $P < 0.0001$) and repeated inoculations (of same-aged leaflets from the same plant with the same fungal inoculum) also led to consistent symptoms ($r_i = 0.70$, $F_{(156,471)} = 10.32$, $P < 0.0001$). Data obtained by different people could therefore be combined for further analyses. Second, to test whether there was an effect of plant age, inoculations using various fungal strains were repeated on the same plant at two different inoculation dates up to six months apart. Repeated inoculations on leaves of the same plant with the same fungal strain at different plant ages led to consistent results ($r_i = 0.59$, $F_{(552,553)} = 3.88$, $P < 0.0001$).

DATA ANALYSIS

The pattern of adaptation was studied for two characters: the infectivity and, for successful infections, the aggressivity of the fungus. Variation in infectivity (presence or absence of infection on leaflets) was analyzed by means of analysis of deviance, based on logistic regression with a binomial error structure; hypothesis testing employed pseudo-F tests, as described in Kaltz et al. (1999). In successful infections, aggressivity had a score between 1 and 3 for each leaflet tested. We analyzed mean aggressivity for each maternal family inoculated with each fungal strain, generating a variable with continuous variation, though this was bounded by 1 and 3. Variation of this variable was analyzed by ANOVA. To normalize the distribution of residuals, parasite aggressivity scores were square root transformed. For both infectivity and aggressivity, statistical models accounted for the nested (populations within species, fungal strains, or plant families within populations) and the crossed (host × parasite) data structure. These models were constructed in analogy to SAS-type II model fitting (SAS Institute 1988). All analyses were carried out with the JMP (SAS Institute 1997) or SAS statistical packages (SAS Institute 1988).

For between-host species analyses (i.e., specialization analysis), we combined infection success and averaged aggressivity scores for each combination of parasite population and host population. In models testing for parasite specialization on the two host species, we first tested for a species × parasite species interaction and, more specifically, for differences between native and nonnative combinations of host and parasite.

For within-species analyses (local adaptation analysis carried out separately for the two host species and their native parasites), we combined values of infection success and averaged aggressivity scores for each combination of parasite strain and plant family. Local parasite adaptation can be defined in two ways (Gandon et al. 1996; Kaltz and Shykoff 1998; Thrall et al. 2002). First, resident parasites perform better on their own host population than do nonresident parasites from other populations ("resident vs. nonresident" comparison); second, parasites perform better on their own than on foreign populations ("home vs. away" comparison). This distinction is relevant only when there are large main effects such that, for example, some parasite populations are highly infectious or some host populations highly resistant (see Thrall

et al. 2002; Kawecki and Ebert 2004). In our statistical models, we repartitioned variation for orthogonal contrasts, testing both types of comparison for each population. For more detailed analysis of the home versus away differences, we specified combinations of parasite and host populations by their geographic distance or the difference in altitude (see Fig. 2). These distances were then used as covariates in analyses of covariance of infection success (taken as arcsine-transformed proportions of infection) with fungal species of origin as an additional explanatory variable. A similar analysis was done for aggressivity.

Finally, two correlations (both at strain and population level) were analyzed. First, we studied the correlation between the degrees of local adaptation in infection success and in aggressivity. The degree of local adaptation was calculated as the difference between the performance of a resident strain and the mean performance of nonresident strains or the difference between the performance of a strain at home and the mean performance of the same strain away. Second, we tested whether the degree of local adaptation (resident-over-nonresident advantage) was correlated with the degree of specialization (native-over-nonnative species advantage). A positive correlation would indicate that local adaptation can reinforce specialization of the parasite.

Results

FUNGAL SPECIALIZATION ON THE TWO HOST SPECIES

For infectivity, we obtained a significant overall difference between host species, with *P. coccineus* less often infected than *P. vulgaris* (see Fig. 3A, Table 1). There was, however, a significant interaction between fungal species of origin and host species, with nearly symmetrically crossing reaction norms. Contrasts revealed fungal specialization on both host species. Parasites collected from

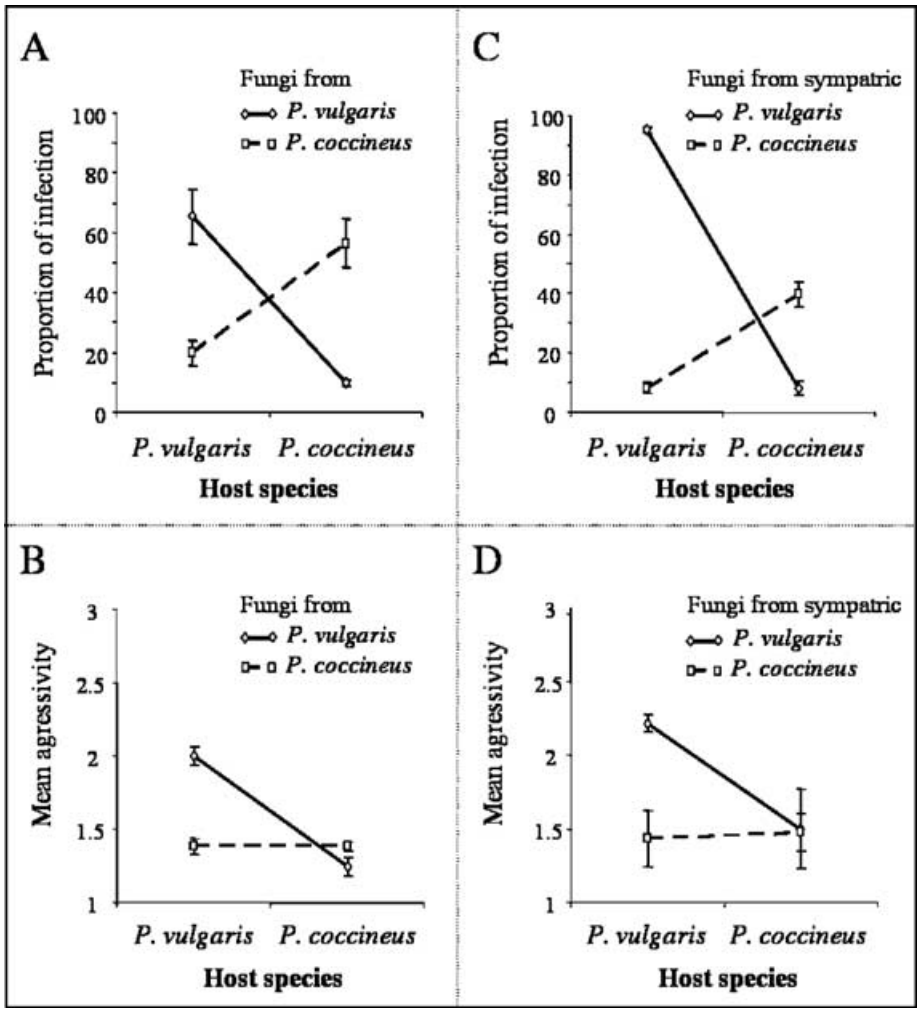

Figure 3. Infection of the two host species, *Phaseolus vulgaris* and *P. coccineus* inoculated with *Colletotrichum lindemuthianum* isolated from either host species. (A) Mean proportion (±SE) of infected plants over all combinations of plant and fungus populations, (B) mean aggressivity (±SE) over all combinations of plant and fungal populations, (C) mean proportion (±SE) of infected plants over sympatric plant and fungal populations, (D) mean aggressivity (±SE) over sympatric plant and fungal populations.

Table 1. Species-level analyses of variation in infection success (analysis of deviance) and aggressivity (analysis of variance). The denominator (Den.) column denotes error terms used for hypothesis testing. Analysis of deviance was based on a logistic regression and mean deviances (MD) were used to calculate quasi-*F* tests. The error term in the analysis of deviance represents the pathogen × host population interaction. The host species × pathogen species interaction for aggressivity was tested against denominator mean squares (MS) and degrees of freedom (df) obtained from a linear combination of several terms (Satterthwaite approximation, Snedecor and Cochrane 1980); for infectivity this correction was not applicable.

			Infection success		Aggressivity	
Source	Den.	df	MD	*F*	MS (10^{-3})	*F*
Host species	(1)	1	69.13	53.2**	92.24	6.16
Pathogen species of origin	(2)	1	0.05	<1	27.37	11.55*
Host species × pathogen species	(a)	1	806.4	135.5**	65.34	14.63*
Host population (host species), (1)	(4)	4	1.30	<1	14.97	3.73
Pathogen population (pathogen host species), (2)	(3)	3	32.80	5.97	2.37	<1
Host species × pathogen pop. (pathogen host species), (3)	(5)	4	5.50	<1	2.68	<1
Pathogen species × host population (pathogen host species), (4)	(5)	3	5.95	<1	4.01	<1
Error, (5)		12	33.48		2.23	

(a) infectivity: (4), aggressivity: (3)+(4)−(5), df = 3.3.
***$P < 0.05$; ****$P < 0.005$.**

P. vulgaris were more infectious on that host than were parasites collected from *P. coccineus* ($F_{(1,3)} = 15.58$, $P = 0.0290$), and vice versa ($F_{(1,3)} = 20.03$, $P = 0.0280$; error term in both contrasts: parasite population [parasite species]). Conversely, native parasites performed better on their own host than on their nonnative host (parasite from *P. vulgaris*: $F_{(1,4)} = 136.2$, $P = 0.0003$; parasite from *P. coccineus*: $F_{(1,4)} = 103.1$, $P = 0.0005$; error terms: host population [host species]).

For aggressivity, we also detected a significant interaction between fungal species of origin and host species (Table 1). However, this interaction (see Fig. 3B) was not as symmetrical as that for infectivity (see Fig. 3A). Fungi isolated from *P. coccineus* showed low (and similar) levels of aggressivity on both hosts, whereas fungi from *P. vulgaris* were very aggressive on their own host species, but not on *P. coccineus*. Consequently, there was a strong and significant native parasite advantage on *P. vulgaris* ($F_{(1,3)} = 57.72$, $P = 0.0047$), and only a small, albeit significant, native parasite advantage on *P. coccineus* ($F_{(1,3)} = 27.43$, $P = 0.0135$; error terms in both contrasts: parasite population [host species]).

We further analyzed fungal specialization exclusively for the population where the two hosts occur in sympatry (VP = CP, see Figs. 3C, D). To this end, we combined infectivity (and averaged aggressivity) over fungal strains (five from *P. vulgaris*, one from *P. coccineus*), so as to obtain one estimate of infection success (or aggressivity) for each combination of parasite origin and plant family. Statistical analysis revealed significant parasite origin × host species interactions for both infection success and aggressivity ($F_{(1,53)} = 357.6$, $P < 0.0001$ and $F_{(1,23)} = 5.32$, $P = 0.0304$, respectively; error terms in both tests: parasite × family [host species] interaction). The patterns of specialization in sympatry (see Figs. 3C, D) were very similar to those across all populations (see Figs. 3A, B).

PARASITE LOCAL ADAPTATION ON THE HOST *P. VULGARIS*

Analysis of both infectivity and aggressivity revealed significant parasite population × plant population interactions (Table 2), indicating differential performance of parasite populations on different host populations. There was a consistent pattern of parasite local adaptation for infectivity (see Fig. 4A). On all three host populations, resident parasites were significantly more infectious than nonresidents; similarly, strains were more infectious on their own ("home") than on allopatric ("away") host populations (see Fig. 4A, Table 4). For aggressivity, we obtained a similar, but less clear-cut pattern (see Fig. 4B). On all three host populations, resident parasites were more aggressive than nonresidents, but none of the three contrasts were statistically significant (see Fig. 4B, Table 4). However, 9 of the 15 strains were more infectious than nonresident strains, resulting in a significant overall pattern of local adaptation at the strain level ($t_{14} = 2.42$, $P = 0.0296$). When comparing "home" versus "away" performance, we detected a significant effect for one of the three populations. Overall, 10 of the 15 strains were more aggressive on their home than away hosts ($t_{14} = 3.18$, $P = 0.0066$).

PARASITE LOCAL ADAPTATION ON THE HOST *P. COCCINEUS*

Unlike on the host *P. vulgaris*, we did not find consistent patterns of local parasite adaptation on *P. coccineus*. First of all, there

Table 2. Population-level analyses of variation in infection success (analysis of deviance) and aggressivity (analysis of variance) on *Phaseolus vulgaris* (with its native pathogen). The denominator (Den.) column denotes error terms used for hypothesis testing. Analysis of deviance was based on a logistic regression and mean deviances (MD) were used to calculate quasi-*F* tests. The error term in the analysis of deviance represents the pathogen strain × host family population interaction. Host population × pathogen population interactions were tested against denominator mean squares (MS) and degrees of freedom (df) obtained from a linear combination of several terms (Satterthwaite approximation, Snedecor and Cochrane 1980).

		Infection success			Aggressivity		
Source	Den.	df	MD	*F*	df	MS ($\times 10^{-2}$)	*F*
Host population	(1)	2	43.94	14.42***	2	29.47	7.29**
Pathogen population	(2)	2	52.66	1.58	2	9.35	.28
Pathogen × plant population	(a)	4	135.31	13.60***	4	21.97	5.13**
Family (host population), (1)	(3)	87	3.05	1.14	87	4.04	1.26
Pathogen strain (path. pop.), (2)	(4)	12	33.35	4.03**	12	33.87	8.43***
Family × pathogen pop. (host pop.), (3)	(5)	174	2.67	2.67***	145	3.21	1.09
Host population × strain (path .pop), (4)	(5)	24	8.28	8.28***	23	4.02	1.36
Error, (5)		583	1.00[(b)]		371	2.97	

(a) infectivity: (3)+(4)−(5), df = 34; aggressivity: (3)+(4)−(5), df = 23. (b) scaled deviance.
***$P < 0.05$; ****$P < 0.005$; *****$P < 0.0005$.**

were no significant parasite × host population interactions for infectivity or aggressivity (see Fig. 5, Table 3). Overall, strains from CF population were more infectious than that from the CP population (see Fig. 5A), resulting in a significant resident advantage in the CF population, and a significant resident disadvantage in the CP population (Table 4). Comparisons between "home" and "away" infection success were nonsignificant for both parasite populations. However, the residuals for the analysis of local adaptation for mean aggressivity on *P. coccineus* were the only significantly nonnormal residuals found in all our analyses. Deviation from normality was due to leptokurtosis, and not asymmetry in the distribution of residuals.

LOCAL ADAPTATION AND GEOGRAPHIC DISTANCE

In the combined analysis of parasites from *P. vulgaris* and *P. coccineus*, performance decreased with increasing geographic or altitudinal distance between host and parasite populations for both infection success and aggressivity (all $F_{1,12} \geq 6.86$, $P \leq 0.0224$). However, these negative relationships were mainly caused by the difference between "home" combinations (distance = 0) and "away" combinations (distances > 0). Across "away" combinations, there were no obvious relationships between geographic distance and fungal performance (all $F_{1,7} < 1$, n.s.), suggesting that the suitability of "away" host populations is not a simple function of geographic distance from the parasite's population of origin.

CORRELATION BETWEEN LOCAL ADAPTATION FOR INFECTIVITY AND FOR AGGRESSIVITY

In four of the five parasite populations, we observed positive values of local adaptation for both infectivity and aggressivity (see Fig. 6). However, correlations between the degree of adaptation for infectivity and aggressivity were not significant (resident vs. nonresident: $r = -0.29$, $N = 15$, $P = 0.2917$ [*P. vulgaris*], $r = -0.32$, $N = 4$, $P = 0.6800$ [*P. coccineus*]; home vs. away: $r = -0.38$, $N = 15$, $P = 0.1655$ [*P. vulgaris*], $r = -0.94$, $N = 4$, $P = 0.0632$ [*P. coccineus*]).

CORRELATION BETWEEN LOCAL ADAPTATION AND SPECIALIZATION

We found positive relationships between local adaptation and specialization. Higher levels of local adaptation (resident advantage) of parasite strains were associated with a stronger native over nonnative species advantage. For infectivity (see Fig. 7A), the correlation was significant for parasite strains from both host species (*P. vulgaris*: $r = 0.62$, $N = 15$, $P = 0.0150$; *P. coccineus*: $r = 0.97$, $N = 4$, $P = 0.0294$). For aggressivity, the correlation was significant only for *P. vulgaris* ($r = 0.93$, $N = 15$, $P < 0.0001$; *P. coccineus*: $r = 0.83$, $N = 4$, $P = 0.1726$), but for both species the degree of specialization was stronger than that of local adaptation (nearly all points above the 45° line in Fig. 7B). We obtained nearly identical correlations (not shown) when calculating the degree of specialization only on allopatric hosts, that is, independently of the performance of a strain as a resident.

Discussion

Our experiment revealed differentiation both at the species level (host specialization) and the within-species level (local adaptation). First, we will treat each level separately, then discuss how adaptive processes at the two levels may interact.

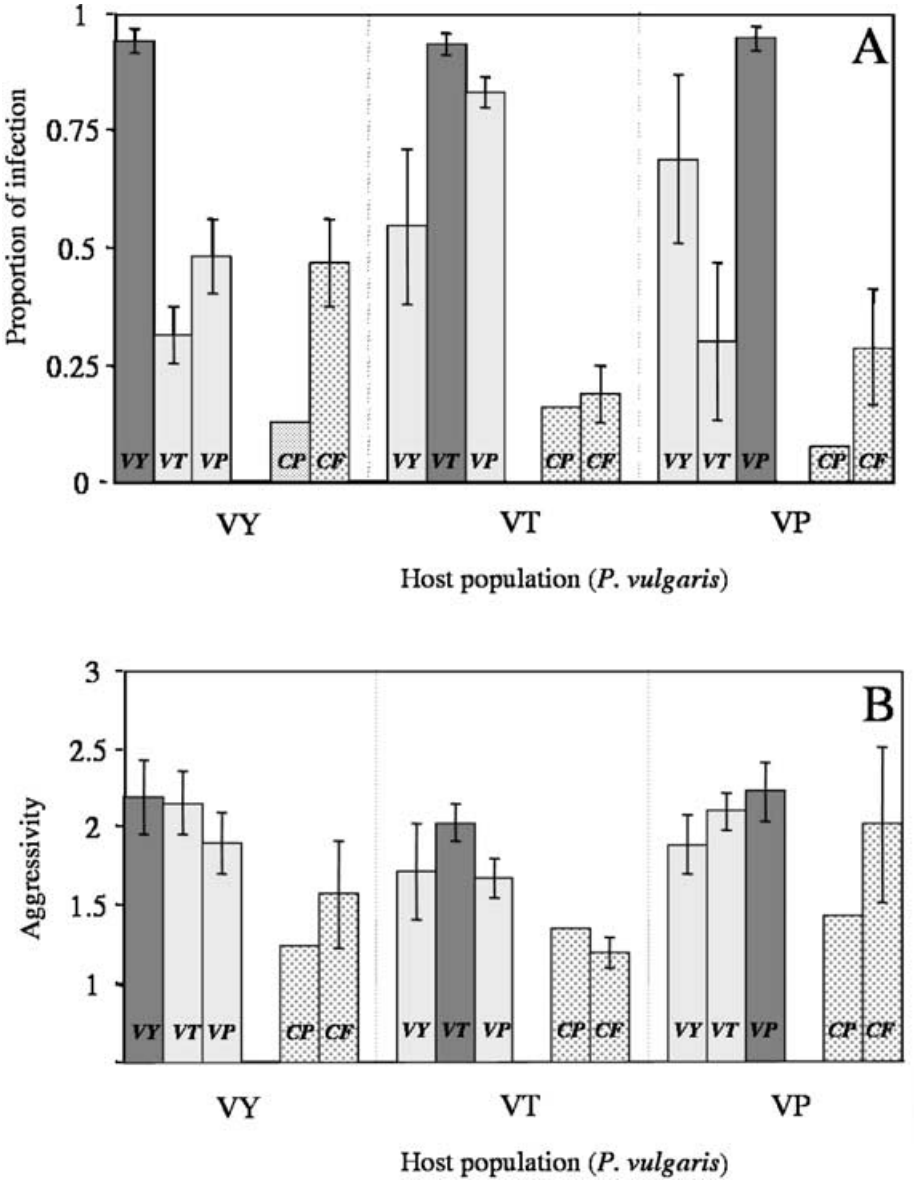

Figure 4. **(A) Mean (±SE) infection success and (B) aggressivity of *Colletotrichum lindemuthianum* isolated from the two host plant species on the three populations of *Phaseolus vulgaris*. Sympatric combinations are in dark gray, allopatric combinations within the same host species in light gray, and cross-species combinations are stippled. Averages were taken over pathogen strains for each combination of pathogen and host population.**

SPECIES-LEVEL EFFECTS AND HOST SPECIALIZATION

Diversifying selection in different habitats is considered a main determinant of ecological specialization (Hutchinson 1959). In parasites and herbivores, adaptation to different hosts is expected to lead to trade-offs in fitness on these different hosts, although such trade-offs are not always found (reviewed by Joshi and Thompson 1995; but also see Via 1991; Craig et al. 1993; Agrawal 2000). In *C. lindemuthianum*, native fungal strains were more infectious than nonnative strains on both hosts (see Fig. 3A), and the crossing reaction norms indicate that strains have adapted to their native host at the expense of their infectivity on the other host. For aggressivity, reaction norms were also crossing, but with a much stronger native-strain advantage on *P. vulgaris* than on *P. coccineus* (see Fig. 3B). This can be interpreted in different ways. The flat reaction norm of parasites from *P. cocccineus* with low aggressivity (and thus low spore production) on both hosts may represent a generalist rather than a specialist strategy. Alternatively, lower overall levels of aggressivity on *P. coccineus* indicate that this species is a less permissive host than *P. vulgaris*. This is consistent with lower overall infection success in our experiment (species effect, Table 1) and lower prevalences in the field

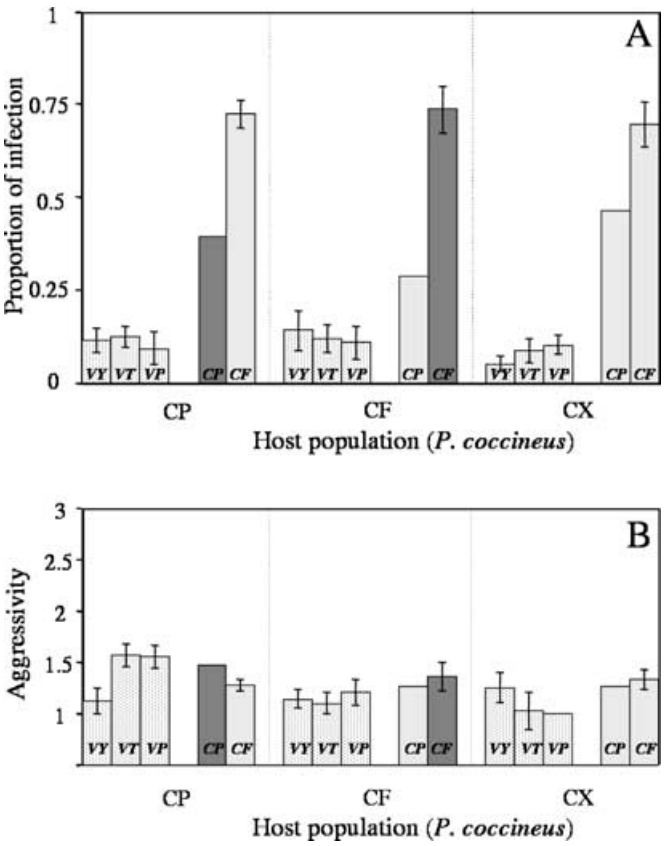

Figure 5. (A) Mean (±SE) infection success and (B) aggressivity of *Colletotrichum lindemuthianum*, isolated from the two host plant species on the three populations of *Phaseolus coccineus*. Sympatric combinations are in dark gray, allopatric combinations within the same host species are in light gray, and cross-species combinations are stippled. Averages were taken over pathogen strains for each combination of pathogen and host population.

(*P. coccineus*: 28% of plants infected; *P. vulgaris*: 45%, D. Sicard, personal observation).

Two host species vary for a multitude of characters. The difference in resistance between two hosts may therefore be related to multiple aspects of their physiology, physical structure, life history, and breeding system. Here, while *P. coccineus* is a perennial predominantly outcrossing species, *P. vulgaris* is an annual mainly selfing species (Delgado-Salinas 1988; Delgado-Salinas et al. 1999). Clearly we cannot know whether differences in reproductive system, rather than other traits, are determinant for the differences in host defense, but to explore this possibility we compare intrafamily correlation coefficients for repeatability. This measures the degree of segregation for resistance phenotypes among individuals with the same mother plant. Repeatability was much lower in *P. coccineus* ($r_i = 0.26$, $F_{(461,929)} = 1.69$, $P < 0.0001$) than in *P. vulgaris* ($r_i = 0.74$, $F_{(560,1238)} = 6.69$, $P < 0.0001$), indicating more variation and segregation of resistance alleles in *P. coccineus*. According to the Red Queen hypothesis, host sexual reproduction and, by extension, outcrossing can generate new resistance genotypes against coevolving parasites. Therefore, outcrossers should evolve resistance more rapidly than selfers (Hamilton et al. 1990; Agrawal and Lively 2001), which could explain why *P. coccineus* is more resistant than *P. vulgaris*. However, other species with contrasting breeding systems should be added to test for this hypothesis.

LOCAL ADAPTATION

Within-species analysis revealed local parasite adaptation for both infectivity and aggressivity in all three populations of *P. vulgaris*. In contrast, in the *P. coccineu*s populations, parasites tended to be either locally adapted or maladapted for infectivity, whereas aggressivity showed no local adaptation at all. In part, the results on *P. coccineus* are difficult to interpret because of the few strains tested. Patterns of host or parasite local adaptation are likely to vary over time and space and may therefore only emerge when averaged over a sufficiently large number of strains or populations (Kaltz and Shykoff 1998; Dybdahl and Storfer 2003).

However, there are several reasons why parasite local adaptation may be more likely on *P. vulgaris* than on *P. coccineus*. First, adaptation to the locally most common host genotypes may be facilitated in populations of the selfing *P. vulgaris*, while outcrossing may enable *P. coccineus* populations to keep track of their coevolving parasites. However, the relationship between host reproductive system and parasite local adaptation is not very clear. Local adaptation has been found for pathogens of selfing *Linum marginale* (Thrall et al. 2002), *Amphicarpaea bracteata* (Parker 1985), and *Triticum aestivum* (Ahmed et al. 1995) but not for those of outcrossing *Podophyllum peltata* (Parker 1989) or *Silene latifolia* (Kaltz et al. 1999). On the other hand, local adaptation also occurs in pathogens of outcrossing *Silene dioica* (Carlsson-Granér 1997) and *Plantago lanceolata* (Laine 2005). Second, local adaptation requires genetically differentiated populations of parasite and host. Indeed, *P. vulgaris* shows significant genetic among-population differentiation (Cattan-Toupance et al. 1998; Papa and Gepts 2003), while *P. coccineus* does not (Escalante et al. 1994). Furthermore, *P. vulgaris* host populations are more strongly genetically differentiated than those of the parasite, indicating more genetic exchange among parasite than host populations (Sicard et al. 1997a,b; Cattan-Toupance et al. 1998). Theory predicts locally adapted parasites if they migrate more than their hosts (Lively 1989; Gandon et al. 1996; Gandon and Michalakis 2002).

Results of tests for local adaptation varied to some degree between tests of resident superiority and home advantage ("resident vs. nonresident" versus "home vs. away"). The correlation between the two comparisons is sensitive to overall differences between parasites or hosts (statistically speaking, to "main effects"). That is, a generally highly infectious strain will be locally adapted relative to nonresidents, but whether this corresponds to

Table 3. Population-level analyses of variation in infection success (analysis of deviance) and aggressivity (analysis of variance) on *Phaseolus coccineus* (with its native pathogen). For further details, see Table 2.

		Infectivity			Aggressivity		
Source	Den.	df	MD	*F*	df	MS ($\times 10^{-3}$)	*F*
Host population	(1)	2	5.5	1.00	2	3.5	<1
Pathogen population	(2)	1	88.8	13.79	1	9.5	<1
Pathogen × plant population	(a)	2	9.1	.07	2	3.6	<1
Family (host population), (1)	(3)	82	163.3	.73	79	19.5	<1
Pathogen strain (path. pop.), (2)	(4)	2	12.9	.88	2	38.6	5.76
Family × pathogen pop. (host pop.), (3)	(5)	67	181.7	2.71***	53	26.4	2.4**
Host population × strain (path. pop), (4)	(5)	4	14.6	3.66*	4	6.7	<1
Error, (5)		59	1.0[(b)]		37	11	

(a) infectivity: (3)+(4)−(5), df = 38; aggressivity: (3)+(4)−(5), df = 24. (b) scaled deviance.
*$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$.

a higher performance on its local host relative to distant hosts depends on whether the local host is generally more or less resistant. Therefore, with strong main effects and only a few populations tested, conclusions about local adaptation can indeed depend on the type of comparison (Thrall et al. 2002; Thrall and Burdon 2003). Nonetheless, we detect local adaptation on *P. vulgaris* for both types of comparison, underlining the robustness of the pattern.

COMPARISON OF INFECTIVITY AND AGGRESSIVITY

Host specialization and local adaptation has been analyzed in diverse host–parasite systems but rarely for more than one character (Ebert 1994; Koskela et al. 2000; Kaltz and Shykoff 2002; Thrall and Burdon 2003). Here we found different specialization patterns for infectivity and aggressivity. Within host species, geographic patterns of adaptation were similar for both characters, although patterns of local adaptation were more clear-cut for infectivity than for aggressivity, possibly because of the restricted resolution of our aggressivity scores. Nonetheless, on both hosts, the degree of local adaptation for aggressivity was not significantly correlated with that for infectivity. Correlations between experimental estimates of local adaptation for infectivity and aggressivity may be determined by complex interactions between the frequency-dependent coevolutionary dynamics, the underlying genetic architecture of the traits, and the relationship between virulence or infectivity and transmission (Dybdahl and Storfer 2003).

Table 4. Summary of tests for pathogen local adaptation for infectivity and aggressivity on five populations of *Phaseolus vulgaris* and *P. coccineus*, based on repartitioning of variation in analyses of deviance or variance (Tables 2 and 3) for contrasts between "resident versus nonresidents" (r/nr) and "home versus away" (h/a). Error terms for infectivity: pathogen strain (r/nr) (13 df); for aggressivity: strain × h/a (4 df). Mean infectivity (arcsine-transformed) or aggressivity (square root transformed) was calculated for each strain on each host population. From these means, local adaptation for each population was calculated as the mean difference (±standard errors of the difference) between residents and nonresidents, or between home and away means, that is, positive values indicate locally adapted pathogens. *P*-values of tests are shown in parentheses.

	P. vulgaris			*P. coccineus*	
	VY	VT	VP	CP	CF
Infectivity					
Resident vs. nonresident	.70±.10 (<.0001)	.32±.11 (.0233)	.60±.18 (.0122)	−.34±.04 (.0397)	.47±.07 (.0748)
Home vs. away	.48±.17 (.0285)	.74±.11 (.0004)	.41±.10 (.0033)	.02±.09 (.8161)	.03±.08 (.7925)
Aggressivity					
Resident vs. nonresident	.04±.07 (.3427)	.09±.05 (.1517)	.05±.05 (.5188)	.06±.02 (.3925)	.03±.04 (.9295)
Home vs. away	.10±.07 (.0078)	−.02±.04 (.2964)	.11±.05 (.0724)	.06±.01 (.1625)	.01±.04 (.6728)

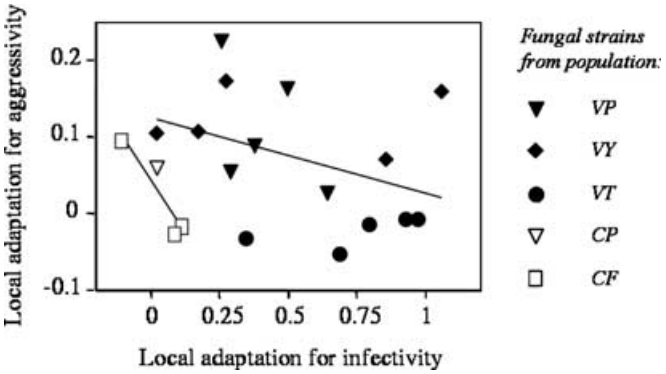

Figure 6. Relationship between local adaptation ("home" vs. "away" performance) for infectivity and local adaptation for aggressivity of pathogen strains from populations of *Phaseolus vulgaris* (black symbols) and *P. coccineus* (open symbols). Degrees of local adaptation were calculated as the differences between the performance of a strain at home and away, positive values indicate locally adapted pathogens.

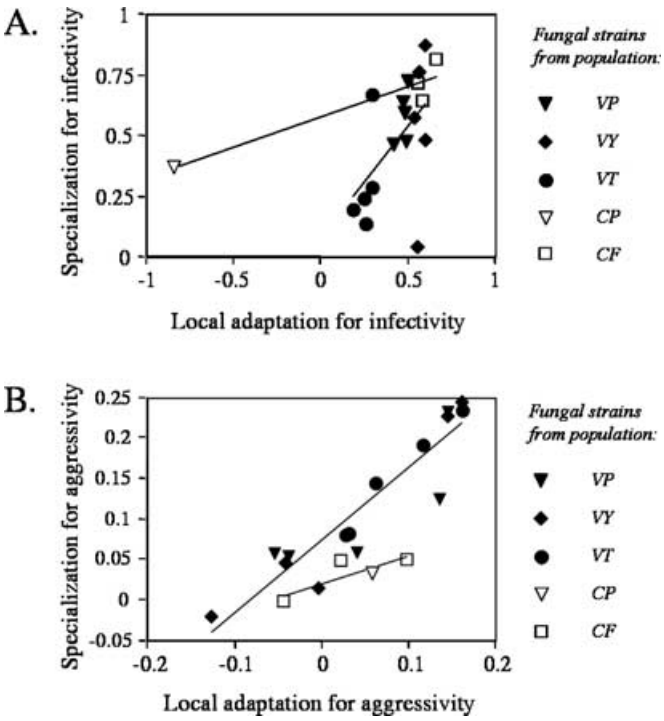

Figure 7. Relationship between the degree of local adaptation ("resident" vs. "nonresident" performance) and specialization (native vs. nonnative species performance) for (A) infectivity and (B) for aggressivity of pathogen strains from populations of *Phaseolus vulgaris* (black symbols) and *P. coccineus* (open symbols). Degrees of adaptation were calculated as the differences between a resident strain and nonresident strains (local adaptation) or a native strain and nonnative strains (specialization), that is, positive values indicate locally adapted or specialized pathogens.

LOCAL ADAPTATION AND SPATIAL STRUCTURE OF FUNGAL SPECIALIZATION

It is often argued that specialization is facilitated by spatial isolation of selection environments that prevents parasite contact with other host environments and with parasites adapted to alternative hosts (Futuyma and Moreno 1988; Mopper and Strauss 1998). However, specialization may depend not only on the geographic separation *between* host species, but also on that of populations *within* host species. In a meta-analysis, Lajeunesse and Forbes (2002) found a negative association between host range and local adaptation and thus concluded that a broad host range limits the potential for local adaptation of parasites. This argument also works the other way around. In subdivided populations, one may see host specialization (and, ultimately, host range) as the consequence of local frequency-dependent selection promoting adaptation to local host populations, rather than to the host species. Thus, local within-species adaptive processes may reinforce between-species specialization (Kawecki 1998). With extreme subdivision, specialization may even be detectable only when parasites are tested on hosts from their own local population.

Here we found both local adaptation and specialization for parasites isolated from *P. vulgaris*. In addition, this fungus performed better on allopatric plant populations of its own host plant species than it did on the other host plant species *P. coccineus* (as presented in Fig. 1D). Therefore, specialization was not the result of local adaptation for the home population alone. The positive correlation between degrees of local adaptation and specialization suggests a common basis of the two. Selection to increase the frequency of favorable alleles in local populations may also increase them at the species level.

We also detected specialization on *P. coccineus*, but local adaptation was not significant (as presented in Fig. 1B) and the relationship between the degrees of local adaptation and specialization was rather flat. This, together with the lower level of population subdivision, suggests that specialization on this host occurs at larger scale than on *P. vulgaris*. Evidence for host specialization across a large geographical scale was also found for the fungal parasite *Microbotryum violaceum* on its several plant hosts. Parasites showed a significant degree of host specificity and neutral markers revealed genetic differentiation among fungal strains from different host species but not by geographic origin (Bucheli et al. 2000).

FROM SPECIALIZATION TO SPECIATION?

Ecological specialization may be a first step toward speciation (Maynard Smith 1966; Felsenstein 1981; Rice 1987; Bush 1994; Schluter 2001; Fry 2003b). Indeed, genetic differentiation among parasite isolates from different host species in several host–parasite systems reveals host race formation (see also Feder et al. 1994; Via 1999; McCoy et al. 2001; Pappers et al. 2002). Allopatry of hosts is the simplest way to ensure species-specific differentiation and genetic divergence. Indeed, in a sympatric population

of two hosts of *M. violaceum,* van Putten et al. (2005) found no significant genetic differentiation between fungal strains from the two hosts. Here, we found strong specialization even in the contact zone. In our system, the habitat range (altitude) of the two host species does not entirely overlap, suggesting that specialization can arise in allopatry. Our study populations are located on a transect, with *P. coccineus* at higher altitudes in the north and *P. vulgaris* at lower altitudes in the south (see Fig. 2). The sympatric population in the middle of the transect may represent a (secondary) contact zone for the parasite host races where specialization is maintained (see Fig. 3), even though the two hosts were entirely intermingled.

Prezygotic mating barriers can prevent recombination between host races even in sympatry. Although the sexual form of *C. lindemuthianum* is not described, low levels of linkage disequilibrium in fungal populations from *P. vulgaris* suggest that sexual reproduction does occur (Sicard et al. 1997b). Thus, if mating occurs on the plant or on host plant debris, it is likely to occur between strains well adapted to the same host. In fact, restriction of mating partners with similar adaptive histories can lead to sympatric speciation via specialization even in the absence of active habitat or mate choice (Giraud et al. 2006). Sequence data of the internal transcribed spacer (ITS) region, used for species identification in fungi, indicate that our strains, although clearly differentiated for selected characters, have not yet diverged for this neutral marker (D. Sicard, unpubl. data). Further study of polymorphism across the whole genome will clarify whether these host-specific strains represent incipient species.

Conclusions

Here we presented one of the first studies testing local adaptation and specialization on different host species for the same parasite. Our results suggest that host specificity of the fungal plant parasite *C. lindemuthianum* on two *Phaseolus* host species occurs at different geographical scales. Specialization on the host *P. vulgaris* seems to be reinforced locally, with fungal strains adapting specifically to their local host populations (as in Fig. 1D), whereas specialization on *P. coccineus* may occur globally without local adaptation of the parasite among populations of this host (as in Fig. 1B). A nonsignificant correlation between the degrees of local adaptation in infectivity and aggressivity suggests that the genetic basis and forces of selection differ between the two traits. Altogether, our results point out the need for simultaneous investigation of spatial variation in different parasite fitness traits for a better understanding of specialization and local adaptation.

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