Correction


The authors note that the legends for Fig. 3 and 4 appeared incorrectly. The figures and their corrected legends appear below.

Fig. 3. Means and SEs of infectivity (proportion of replicate plants infected) for four different treatments. WS, same host, same location; WD, different host, same location; BS: same host, different location; BD, different host, different location. All pairs of treatments are significantly different (P < 0.001) except WS-BS in post hoc Tukey-Kramer comparisons.

Fig. 4. Model of evolutionary changes in pathogen and host specificities leading to high host specificity in the pathogen. αi and βj represent genotypes of two pathogen elicitors. Ai and Bj represent genotypes of R-genes in a given host. The genes in the three hosts are not necessarily orthologous. Subscripts represent specificities. Pathogen genotypes are diploid, but are shown as haploid for convenience and should be interpreted as homozygous at each locus. If the subscript of α matches the subscript of Ai, or if the subscript of β matches the subscript of Bj, then the pathogen is avirulent (plant is resistant). Otherwise the pathogen is virulent (plant is susceptible). A–F represent successive evolutionary changes. The specific changes are indicated by red subscripts. Pathogen genotypes in red indicated genotypes introduced to the community either by immigration or mutation. Pathogen genotypes directly above host genotype are virulent on that host. (A) No pathogens are originally present, and a new pathogen genotype is introduced by immigration. This genotype is virulent on all host species. (B) Host species 1 and 3 evolve resistant genotypes, leading to avirulence of the pathogen on these hosts. (C) A new pathogen genotype immigrates or is produced by mutation. (D) Evolution of a novel resistance allele in Host 2 makes the new resistant to the new pathogen genotype. (E) A new pathogen genotype immigrates, and is initially virulent on all three host species. (F) Host species 1 and 2 evolve new resistance genotypes, making the new pathogen genotype avirulent. At this stage, the three pathogen genotypes are each virulent on only one host species. Table S2 shows that pathogen genotypes derived from these by recombination are also all virulent on at most one host species.

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Evolution of host range in *Coleosporium ipomoeae*, a plant pathogen with multiple hosts

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Plants and their pathogens coevolve locally. Previous investigations of one host-one pathogen systems have demonstrated that natural selection favors pathogen genotypes that are virulent on a broad range of host genotypes. In the present study, we examine a system consisting of one pathogen species that infects three host species in the morning glory genus *Ipomoea*. We show that many pathogen genotypes can infect two or three of the host species when tested on plants from nonlocal communities. By contrast, pathogen genotypes are highly host-specific, infecting only one host species, when tested on host species from the local community. This pattern indicates that within-community evolution narrows the host breadth of pathogen genotypes. Possible evolutionary mechanisms include direct selection for narrow host breadth due to costs of virulence and evolution of *ipomoea* resistance in the host species.

Much of plant-pathogen coevolution is mediated by “gene-for-gene” (GFG) interactions. These interactions involve R genes in plants and corresponding virulence/avirulence genes in the pathogen (1). At a given pair of corresponding loci, a host may carry either a resistant (*Res*) or a susceptible (*Sus*) allele, or both, with *Res* typically being dominant. The pathogen may carry either a virulent (*Vir*) allele or an avirulent (*Avr*) allele. Infection results, unless at one pair of corresponding loci, the plant R locus has a *Res* allele and the pathogen has an *Avr* allele. Models of the evolution of GFG systems generally predict that generalist pathogens (those able to infect multiple host-resistance genotypes) will be favored by natural selection over highly specialized genotypes that can infect only one resistance genotype (2–6). Experimental analyses of pathogen host breadth in natural plant-pathogen systems are consistent with these expectations in that pathogen isolates are generally able to infect multiple host-resistance genotypes, especially in host populations with high levels of resistance (7–10).

With very few exceptions (11, 12), the evolution of pathogen host range has been examined, both theoretically and empirically, for a single pathogen species interacting with a single host species. Many pathogens, however, are capable of infecting multiple host species. Predictions of evolutionary models based on a single evolving host species cannot be clearly extrapolated to this situation. Moreover, there are reasons to believe that, with multiple host species, selection for generalism may not be as prevalent. Maintaining infectivity on multiple hosts requires continued success in the coevolutionary arms race with more than one independently evolving host genome. The conditions under which this maintained infectivity can occur are likely more restrictive than with only one host, although this possibility has not been examined theoretically. In addition, selection to maintain infectivity on a particular host is likely weaker when the pathogen population can successfully reproduce on another host (see ref. 13 for an analogous argument with respect to partial resistance). Finally, costs associated with the ability to infect multiple host species (e.g., ref. 14) are likely greater than costs associated with the ability to infect multiple genotypes within the same host. All of these factors would tend to weaken selection for a broad host range and thus promote the evolution of specialist pathogen genotypes within populations.

One approach to determining whether there is an evolutionary tendency for host breadth to be narrowed within populations is to compare pathogen host breadth in its local native community with host breadth on hosts from outside its native community (e.g., refs. 9 and 13). The latter constitutes an estimate of host breadth on host species with which the pathogen has presumably not recently coevoluted and is also an estimate of host breadth for a pathogen strain that has recently immigrated into a new community. If evolutionary processes within local communities act to promote specialization, host breadth should be lower on hosts from the native community. In this report, we demonstrate that this pattern is exhibited for a host–pathogen system consisting of one pathogen and three host species.

Methods

Ethics Statement. No specific permits were required for the collections used in these experiments. Some collections were made in public road rights-of-way, and where collections were made from private land, permission to do so was granted by landowners. No endangered or protected species were affected by this work.

The *Ipomoea–Coleosporium Pathosystem*. Throughout the eastern United States, several morning glory (*Ipomoea*) species are alternate hosts to a red rust, *Coleosporium ipomoeae*. Among these species are *Ipomoea coccinea* L., *Ipomoea hederacea* Jacq., and *Ipomoea purpurea* (L.) Roth, each of which is an annual herbaceous flowering plant that occurs commonly in agricultural fields, field margins, and other disturbed habitat. Outcrossing rates in these species vary: *I. hederacea* is highly selfing (93% (15)), *I. purpurea*’s selfing rate has been reported to be between 20% and 70% (15–17), and, although selfing in *I. coccinea* has not been explicitly quantified, the species is self-fertile (18). None of the species are capable of hybridizing.

Throughout the eastern United States, the rust *C. ipomoeae* attacks several species of morning glory hosts, including *I. hederacea*, *I. purpurea*, and *I. coccinea*. The pathogen is a heteroecious rust, with pines (especially *Pinus taeda*) in the southeastern United States as its primary host. In early summer (late May through early June), dikaryotic spores (aeciospores) are shed from inoculated pines. The spores are carried by the wind to host plants, where they germinate and produce teliospores, which become aeciospores when the spores are exposed to light. The aeciospores infect the host plant and produce dikaryotic endospores, which become teliospores when exposed to light. The teliospores are wind-borne and infect pine trees, where they develop into aeciospores, completing the life cycle. The pathogen system consists of one pathogen species and three host species, and is consistent with these expectations in that pathogen isolates are generally able to infect multiple host-resistance genotypes, especially in host populations with high levels of resistance (7–10).

Significance

Patterns of host breadth evolution in pathogens that attack multiple host species have seldom been quantified. Previous investigations of pathogens attacking a single host indicate that pathogen genotypes evolve a broad host range across host genotypes. By contrast, this investigation demonstrates that, in a pathogen attacking several host species, pathogen genotypes evolve to be highly host-specific. This difference suggests that the evolutionary dynamics of pathogen host breadth may differ depending on whether the pathogen attacks only one or multiple host species.

Author contributions: T.M.C. and M.D.R. designed research, performed research, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

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formed on pines and subsequently infect the Ipomoea secondary hosts. Infections of Ipomoea produce asexual spores (urediniospores) that infect the secondary hosts, with up to 15 asexual generations occurring during the summer. In the fall, asexual spores (teliospores) colonize the primary hosts and subsequently undergo diplotization, meiosis, and fusion to form the dikaryotic hyphae that give rise to the aeciospores in the spring.

Crossing experiments have shown that resistance to individual pathogen inocula (spores collected from the same host species at a particular site) is often determined by genotype at a single genetic locus (19, 20). In addition, these studies provided evidence indicating that loci conferring resistance to a particular pathogen inoculum sometimes differ between populations of the same host species. These results suggest that GFG interactions may frequently be involved in the co-evolution of C. ipomaeae and its hosts. In addition, infection by C. ipomaeae reduces fitness substantially in I. purpurea (21, 22), suggesting the potential for the pathogen to drive the evolution of resistance in its hosts.

Field Censuses. Sites containing at least one of the three focal morning glory species in North Carolina were censused to determine the natural distribution of infection during the years 2006–2008 (Fig. 1). Each year, we visited these communities during the early summer after plant germination had occurred and recorded which Ipomoea species were present. During repeated visits over the summer, we assessed the proportion of plants of each species harboring infections. By late August, these proportions fell into two discrete categories: >90% of plants infected or <10% of plants infected.

Experimental Assessment of Compatibility. To investigate the pattern of host specificity for pathogen genotypes, we performed a series of cross-inoculations in the laboratory between pathogens collected from a single host species at a particular location, and host plants of each species at that and other locations. The set of inoculations performed represented a compromise between complete coverage of all hosts and pathogens at a given location and coverage of as many locations as possible. Individual inocula were collected from multiple plants of the same species at a given site because propagation of single-spore isolates was not possible. They thus may represent multiple pathogen genotypes. There are two advantages to this approach: (i) it reduces the possibility of attributing an observed compatibility reaction to a sample not representative of the majority pathogen genotype associated with a given host population; and (ii) it decreases the probability of not including minority pathogen genotypes in cross-inoculations. One potential disadvantage is that it may confound the host ranges of different pathogen genotypes. However, this possibility is a problem only if genotypes in an inoculum have different host specificities. As described below, this possibility can be detected because genotypes able to infect a given host yield visible pustules, whereas genotypes unable to infect that host yield visible signs of a hypersensitive response. Trials yielding both responses on the same plant were very rare (see below).

Seeds from mature plants were collected in August through September of the years 2006–2007, haphazardly and with 3 m between collections to avoid repeated collection from single plants. Assessment of compatibility for collected seeds was carried out in the year after collection, such that the inoculum encountered by experimental plants was that which these plants would have encountered in the field after germinating 1 year after seed dispersal.

Two field locations (CRG and LF) were chosen for complete reciprocal cross-inoculation, and three locations (CB, CL, and MO) were chosen for additional cross-inoculations. Because not all host–pathogen combinations from these additional locations could be tested due to space limitations, a subset of combinations was chosen at random. Each cross-inoculation trial represents a combination of plants from one host population (one Ipomoea species at one location) with a spore inoculum collected from one host species either at the same location or a different location. An average of 12 plants per host species–site combinations were planted to be used as replicate hosts for each trial. Each plant was the progeny of a different maternal plant. Several trials were conducted once during one year, and again during a later year, to assess repeatability of results. Plants used in the inoculation trials were grown for 14 d in the Duke University Greenhouse in fertilized soil (14–14–14) and were watered every other day. Experimental plants were randomly placed into blocks of 36 and grown in identical 36-pot cell packs, each in one greenhouse tray. At 14 d, plants were moved to a climate-controlled growth room with a 16-h photoperiod and a temperature regime of 16 °C and 30 °C at 22 °C. At 21 d, each 36-plant group was administered an inoculum consisting of a collection of urediniospores from one host species at one location.

Urediniospores were collected from the field in the early summers of 2007 and 2008, soon after infections became visible in field populations. They thus represent the first generation of spores produced on the secondary hosts. Pustule-infected leaves were removed from plants and placed in airtight bags for transport. In the laboratory, spores were washed from live pustules with distilled water, and the resulting spore suspension was diluted to a standard 2,000 particles per mL. Controlled inoculation was carried out by first saturating soil and plants with water 8 h before the end of the light stage of the photoperiod. A clear plastic dome 8 inches (20 cm) high was placed over plant trays at that time to elevate relative humidity and simulate natural conditions in the field at dusk. A total of 5 mL of a standardized spore suspension was then applied via a spray bottle to the undersides of experimental plant leaves and the plants were left undisturbed for 7 d before domes were removed. Plants were observed daily from age 28 to 35 d for scoring; plants on which orange uredia appeared were scored as infected. Plants showing the hypersensitive response, as indicated by the appearance of black flecks or spots on leaves, were scored as resistant.

Statistical Analyses. The primary response variable was the proportion of replicate plants infected for a particular inoculum, which was arcsine square-root transformed before analysis. Inocula were classified by (i) whether or not the host species inoculated was the same as the species from which the pathogen inoculum was collected; and (ii) by whether the host inoculated was or was not from the same site as the site from which the inoculum was collected. A two-way analysis of variance with interaction was performed with these two categories by using the GLM procedure of the SAS system (Version 9.2) (23). Mean and SE for each treatment were calculated on untransformed proportions by using the MEANS procedure of the SAS system.

For purposes of distinguishing among pathogen genotypes, we constructed an “infectivity vector.” Each element of the vector corresponded to a particular host species–location combination. Each element was scored as a 0 or 1 based on whether proportion of replicate plants infected was >0.5 or <0.5 (see below). Because not all inocula were tested on all host–location combinations, some elements were un-scored in each vector. Two microblot assays were considered to represent different infectivity genotypes if, for at least one vector element, one inoculum had a 1 and the other had a 0. Similarly, within each host species, resistance genotypes were distinguished by constructing an analogous “resistance vector,” each element of which indicated resistance (0) or susceptibility (1) to a different pathogen inoculum. Resistance genotypes for a particular host species from two different locations were considered different if for at least one vector element, one vector had a 0 and the other had a 1.

Results

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

Experimental Assessment of Compatibility. Inoculations of individual plants generally resulted in one of two outcomes: Either a plant became infected, as indicated by the presence of sporulating uredia, or it resisted infection, as indicated by lack of uredia and the presence of small regions of necrotic tissue resulting from the hypersensitive response. Only 3 of the 1,137 observed experiments in which plants became infected and also exhibited the hypersensitive response, suggesting that only very rarely were plants inoculated with a spore collection that included genotypes with two different specificities. Although we have not yet characterized the genetics of these interactions, this all-or-nothing response is typical of GFG interactions (1, 24).

The mean absolute difference in proportion infection between inoculations replicated across years was 0.089 (± 0.040 SE). When the one replicate that was 0.00 in 2007 and 0.67 in 2008, which may represent a change in infectivity or resistance, is dropped, this difference is 0.060 (± 0.029). These differences are small compared with differences in treatment means (Table S1), indicating that results are generally replicable across years.

The proportion of trials in which all replicate plants exhibited the same outcome was 0.81. In the remaining trials, most plants showed one outcome, whereas a few showed the other, resulting in a strongly bimodal distribution of outcomes (Fig. S1). This pattern allowed us to categorize the host population represented in a trial as either preponderantly susceptible or preponderantly resistant to a particular pathogen isolate, with the former having <50% of plants infected and the latter having >50% of plants infected. These results indicate that, although resistance or susceptibility to particular pathogen isolates is nearly fixed in host populations, minority resistance genotypes may exist.

Comparison of patterns of infectivity across all host species × population combinations (infectivity vectors and resistance vectors) for different inocula reveals extensive genetic variation for infection outcome, both within the pathogen and within each host species (Fig. 2 and Figs. S2 and S3). Among the 12 inocula tested, 11 had unique patterns of infectivity that differ from all other inocula, except for CB-H, which could not be distinguished from the patterns for 5 other inocula (Fig. 2A). There are thus minimally 11 distinct patterns of infectivity and, hence, minimally 11 distinct pathogen genotypes, among the 12 inocula. Similarly, within I. hederacea, I. purpurea, and I. coccinea, there were distinct patterns of infectivity across pathogen inocula: For I. hederacea, there were minimally 4 patterns among 5 populations tested; for I. purpurea, there were 2 patterns among 4 populations tested; and for I. coccinea there were 2 patterns among 4 populations tested (Fig. 2B). This variation indicates that populations of hosts and pathogen have diverged genetically among locations with respect to pathogen inoculum-specific resistance.

The set of 100 cross-inoculations can be broken down into four categories (Tables S1–S3): WS (within location, same host), inoculation of the same host at the same location from which the inoculum was collected; WD (within location, different host), inoculation of a different host at the same location (blue squares); BS (between locations, same host), inoculation of the same host from a different location (tan squares); and BD (between locations, different host), inoculation of a different host from a different location (white squares). Category WS represents a control for the inoculation procedure, because it involves combinations of host species and pathogen strain for which infection has previously occurred. Collectively, categories BS and BD represent the average number of host species infected at other locations, which corresponds to the expected host breadth of the pathogen inoculum if it were introduced into a new community. Finally, categories WS and WD collectively represent the average host breadth of an inoculum after the pathogen has interacted with the local host community for a period. Under the hypothesis that evolution resulting from interactions within a community does not alter pathogen host breadth, the average host breadth ascertained from categories WS and WD should be the same as that ascertained from categories BS and BD.

Mean infectivity in the control trials (WS) was 0.945 (Table S1 and Fig. 3), indicating that our infection procedure was highly successful. Infection frequencies were higher for inoculation of the same host species than for inoculation of different host species, regardless of whether they were from the same or different locations (WS and BS) (Table S1). In addition, although there was a substantial probability (0.523) of infection for inoculation of different hosts at different locations (BD), no infections occurred for different hosts at the same location from which the inoculum was collected (WD) (Table S1 and Fig. 3).
An analysis of variance indicated that the effects of both host species (same or different) and location (same or different) were highly significant, as was the interaction (Table S2). Post hoc comparisons indicated that all pairs of treatments, except for WS-BS, are statistically significant ($P < 0.008$; Table S3). The lack of significance for WS-BS indicates that there is no evidence that inocula tested on the host from which they were collected differ in activity on the local and nonlocal hosts. By contrast, the high significance of the WD-BD comparison indicates that in infection of hosts that differ from the collection host, infectivity is much higher when the inoculated host is nonlocal than if it local. Thus, although inocula from each host species are able to infect other host species from other locations, they are unable to infect other host species from the native location. On average, inocula tested on hosts from nonnative communities are able to infect an average of 1.83 host species per community (including the species from which the inoculum was collected); by contrast, when tested on hosts from their native community, inocula are able to infect only 1 host species.

This pattern also appears to hold for when inocula collected from different host species are analyzed separately (Tables S1–S3). In each case, WS and BS exhibited the highest mean infectivities and do not differ from each other. In addition, infectivity was significantly lower for WD compared with BD, with infectivity on a different, nonlocal host ranging from 0.424 to 0.641, whereas infectivity on a different local host was uniformly 0.

Discussion

The primary result of our cross-inoculation experiment is that pathogen host breadth is narrower when tested on hosts from the pathogen’s native community than when tested on hosts from nonnative communities. In particular, although a pathogen inoculum can infect only the species it was collected from in its native community, it can typically infect both that species and others from nonnative communities. This pattern implies that evolution within local communities narrows pathogen host breadth. It also conflicts with both theoretical expectations for, and empirical observations on, plant pathosystems consisting of one host and one pathogen species: Models predict the evolution of broad host range across host genotypes (2–6), and empirical studies are generally consistent with this prediction (7–10).

Several possible mechanisms could explain the narrowing of host breadth in local populations. Here we outline four and argue that two constitute unlikely explanations. One evolutionary process that could reduce host breadth is selection on the pathogen strain for avirulence on host species it initially infected. Such selection could be driven by costs of virulence. In particular, consider a new pathogen strain that migrates into a community. Our results suggest that, initially, that strain would be virulent on two or more local hosts. A mutation that caused the pathogen to become avirulent on one of the hosts would presumably incur a strong fitness penalty because mutant spores landing on that host would be essentially dead. As described above, colonization of morning glories in the spring and early summer occurs by a “rain” of aeciospores from pine trees, which means that the probability of any particular mutant spore landing on a particular host species is approximately proportional to the abundance of that host species in the community. If the three host species are approximately equally abundant, then the mutant would incur a fitness decrement of 1/3–1/2, depending on whether the pathogen initially infected three or two hosts, respectively. It is presumably this type of selection that has led to the persistence of pathogen genotypes that attack multiple resistance genotypes in systems with only one host species (7–10).

In order for the mutant to increase in frequency, costs of virulence would have to offset this increased mortality. Such costs would have to be very large. Although costs of virulence appear to be frequent (25), the few studies that have quantified costs in fitness units have found costs ranging up to ~0.25 (26–28), which would not be sufficient to offset the mortality costs described above. Moreover, our observation that most inocula exhibit virulence to nonnative hosts suggests that costs of virulence are typically small in this system. Nevertheless, without actually measuring these costs, we cannot rule out the possibility that some of them are substantial enough to compensate for the increased mortality of a mutant that reduces host breadth.

Under this scenario, selection would favor the mutant because of higher reproductive success on the host(s) it can infect. Passage through these hosts would increase the frequency of the mutant, which more than compensates for the reproductive success of the nonmutant on the host species the mutant does not infect. It is also possible that another mutation could arise that caused avirulence on a different host species. If the cost of virulence on that host were sufficiently high, this mutation would also increase in frequency. To the extent that pathogen populations are regulated independently on different hosts, two specialist mutant strains could persist, each with a narrower host range than the original strain, which would be eliminated (29). An alternative possibility is that selection against unnecessary virulence occurs on the primary host during the winter and spring.

A second, related explanation for our results is that they are an artifact of the time during the season when pathogens were sampled: Mating and recombination on the primary host generates genotypes that can infect several host species, but asexual passage through individual secondary hosts selects for specialists by the end of the summer. In other systems, passage experiments of pathogens on particular hosts frequently results in loss of virulence on other hosts, presumably because of costs of virulence (30). There is certainly the potential for this type of selection for specialization because C. ipomoeae can cycle through as many as 10–15 asexual generations on secondary hosts. This explanation seems unlikely, however, because we collected inocula at the very beginning of the season before asexual passage. Host specificity is thus present immediately after sexual reproduction and is not an artifact of measuring host breadth at the end of the summer season.

A third explanation for the existence of host–specialist pathogen genotypes within local populations is that resistance and virulence are determined not by a GFGs system but by some other mechanism. One possible mechanism is that the pathogen produces two potential genotypes that could explain the narrowing of host breadth in local populations. Here we outline four and argue that two constitute unlikely explanations. One evolutionary process that could reduce host breadth is selection on the pathogen strain for avirulence on host species it initially infected. Such selection could be driven by costs of virulence. In particular, consider a new pathogen strain that migrates into a community. Our results suggest that, initially, that strain would be virulent on two or more local hosts. A mutation that caused the pathogen to become avirulent on one of the hosts would presumably incur a strong fitness penalty because mutant spores landing on that host would be essentially dead. As described above, colonization of morning glories in the spring and early summer occurs by a “rain” of aeciospores from pine trees, which means that the probability of any particular mutant spore landing on a particular host species is approximately proportional to the abundance of that host species in the community. If the three host species are approximately equally abundant, then the mutant would incur a fitness decrement of 1/3–1/2, depending on whether the pathogen initially infected three or two hosts, respectively. It is presumably this type of selection that has led to the persistence of pathogen genotypes that attack multiple resistance genotypes in systems with only one host species (7–10).

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Model of evolutionary changes in pathogen and host specificities leading to high host specificity in the pathogen. $\alpha_i$ and $\beta_j$ represent genotypes of two pathogen elicitors. $A_i$ and $B_j$ represent genotypes of R genes in a given host. The genes in the three hosts are not necessarily orthologous. Subscripts represent specificities. Pathogen genotypes are diploid, but are shown as haploid for convenience and should be interpreted as homozygous at each locus. If the subscript of $\alpha$ matches the subscript of $A$, or if the subscript of $\beta$ matches the subscript of $B$, then the pathogen is virulent (plant is susceptible). Otherwise the pathogen is avirulent (plant is resistant). $A$–$F$ represent successive evolutionary changes. The specific changes are indicated by red subscripts. Pathogen genotypes in red indicate genotypes introduced to the community either by immigration or mutation. Pathogen genotypes directly above host genotype are virulent on that host. (A) No pathogens are originally present, and a new pathogen genotype is introduced by immigration. This genotype is virulent on all host species. (B) Host species 1 and 3 evolve resistant genotypes, leading to avirulence of the pathogen on these hosts. (C) A new pathogen genotype immigrates or is produced by mutation. (D) Evolution of a novel resistance allele in host 2 makes host 2 resistant to the new pathogen genotype. (E) A new pathogen genotype immigrates, and is initially virulent on all three host species. (F) Host species 1 and 2 evolve new resistance genotypes, making the immigrating pathogen genotype avirulent. At this stage, the three pathogen genotypes are each virulent on only one host species. Table S2 shows that pathogen genotypes derived from these by recombination are also all virulent on at most one host species.

One potential limitation of our study is that we have inferred that inocula consist of multiple genotypes, those genotypes have the same specificities. This inference is based on our failure, except in three cases, to find both successful infection and evidence of a hypersensitive response on the same plant. This inference may be suspect if successful infection by one genotype facilitates infection by a normally avirulent genotype or if a hypersensitive response results in a normally virulent genotype not infecting a plant. Studies on other pathogens have documented
priority effects, in which, for example, inoculation by a virulent pathogen facilitates infection in a subsequent inoculation by a normally avirulent pathogen (41–43). However, when a virulent and an avirulent pathogen are simultaneously inoculated, such priority effects are generally absent (44–46). Because in our study different genotypes in an inoculum were introduced to a plant simultaneously, it seems unlikely that there would have been sufficient time for defenses to be activated to prevent the least some infection by the virulent genotypes. Similarly, it seems unlikely there would have been time for infections to produce biochemical changes that would completely prevent a hypersensitive response to the avirulent genotypes.

More important, however, is that even if inocula normally contained multiple genotypes with different specificities, our conclusions would not be substantially altered. Consider an inoculum collected from host A at site 1 that successfully infects hosts A and B at site 2, and suppose this pattern is because the inoculum contains two genotypes: one that infects host A at site 2 and one that infects host B at site 2 (they both infect host A at site 1). However, because the second genotype infects host B at site 2, the expected probability that it also affected host B at site 1 when it first arrived at site 1 was about 0.83 because it represents a category BS interaction. However, this pattern is never the case (i.e., a genotype from host A at site 1 never infects host B at site 1), implying that local evolutionary change in this genotype causes it not to infect host B; its host breadth is narrowed. The processes that could produce this narrowing are the same as those described above.

Theoretical investigations have suggested repeatedly that coevolutionary interactions between plants and pathogen species will tend to produce pathogen genotypes that infect multiple host genotypes. In general, empirical investigations have supported these predictions. These results might be extrapolated to suggest that, in communities with multiple host species, selection should similarly lead to the persistence of pathogen genotypes with a broad host range. However, we have found just the opposite. Determining whether selection directly favors the evolution of host-specific genotypes in C. ipomoeae or, rather, favors genotypes with a broad host range but is counteracted by the evolution of resistance in the hosts will require both determining the magnitudes of costs associated with virulence as well as whether increased resistance to specific pathogen genotypes evolves in local communities.

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