Stable Polymorphisms in a Two-Locus Gene-for-Gene System

J. Segarra

Department of Plant Production and Forest Science, ETSEA, University of Lleida, Av. Alcalde Rovira Roure 177, 25198 Lleida, Spain.
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ABSTRACT

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A two-locus gene-for-gene model is presented to analyze coevolutionary dynamics in interactions between host plants and their pathogens. Using both analytical and simulation approximations, we show that the behavior of the model is very simple with one locus. In the reciprocal genetic feedback version, there is a smooth outward spiral toward the boundaries. In the delayed feedback version, there is an infinite family of closed curves corresponding to different initial conditions. Both versions of the model are stabilized by the addition of recurrent mutation. Either a stable interior equilibrium or a stable limit cycle appears. But with the two-locus model, different coevolutionary outcomes are predicted according to the parameter values. For a wide range of small and medium values

of virulence and resistance costs, complex fluctuations arise. The number of virulence alleles per isolate and the number of resistance alleles per plant cycle indefinitely. If the costs of both virulence and resistance are above a threshold, the final state of the coevolutionary dynamics is a stable single-resistance static polymorphism in the host and avirulence in the parasite. An equivalent threshold to maintain a disease-free host population was obtained analytically for a multilocus system. These expressions can be used to determine the number of single-resistance host genotypes that would have to be present in a mixture to prevent the spread of any virulent race of pathogen. The model demonstrates that it is preferable to use mixtures of single-resistant genotypes rather than using multiple resistance alleles in the same cultivar.

Additional keywords: epidemiology, population genetics.

In over 40 plant-parasite associations it has been demonstrated or suggested that the resistance of the plant and the virulence of the pathogen interact as gene-for-gene systems (9,29). These associations include a broad range of plant and pathogen taxa and life histories. The plants include both annual and perennial crops. The pathogens include fungi such as *Venturia inaequalis* and *Pyricularia oryzae*, bacteria such as *Xanthomonas campestris*, and viruses including *Tobacco mosaic virus*. The crop diseases include such important ones as rusts, mildews, and bunts of cereals. These gene-for-gene systems at a population level are characterized as highly polymorphic, with the appearance of boom and bust cycles and virulence being much more frequent than resistance (6,18). This gives the pathogen population a capacity for quick adaptation to the crop's resistance structure and, by extension, to different control measures. Most of the genetic information currently available is for agricultural pathosystems (5,23). More recently, the complexity and diversity of the virulence and resistance in natural pathosystems has been characterized (7).

Different types of mathematical models have been formulated to understand the coevolutionary process of the gene-for-gene systems. On one hand, there are the purely genetic models with frequency-dependent selection (14,16). On the other, there are the ecological genetic models that explicitly model the population size. As well as the genetic parameters, these models also include ecological (10) or epidemiological parameters (2,22). In this latter view, the model is formulated in terms of the epidemiology of the disease and virulence arises as a consequence of a parasite's adaptation to maximize its total transmission in the host population. This is generally expressed in terms of the basic reproductive number, R_0 , of the infection caused by the parasite (8,11). The definition of R_0 is the expected number of new infections caused

by a typical infected individual during its period of infectiousness in a population only consisting of susceptible. Nowadays, more complex and realistic models have been formulated to study conditions under which new resistance and virulence genes evolve and persist, and the factors that maintain genetic polymorphisms, such as host and spatial heterogeneity, annual or seasonal epidemics, and multilocus genotypes (30).

In 1977, Leonard (16) proposed a gene-for-gene model to account for coevolution of resistance and virulence in natural plant diseases. This model predicts low resistance and high virulence equilibrium frequencies similar to those found in plant pathogen systems. However, the equilibrium point (EP) is locally unstable and stable polymorphisms could not develop. Leonard (17) showed that host-parasite gene-for-gene dynamics often produce unstable cycles so far from the EP that genes for virulence and resistance will always enter the population at frequencies within the limit cycle. In those cases, balanced polymorphisms are originated in biological terms. One factor that appears to promote the stability of the virulence and resistance polymorphisms is the introduction of various loci. Sasaki (25) formulated a multilocus gene-for-gene model, where both host and parasite populations can maintain polymorphism in each locus and retain complex fluctuations for low virulence and resistance costs. In the same way, a matching alleles model with two loci produce sustained complex cycles and even apparently chaotic dynamics (26). Recently, Leonard's model has been analyzed in depth for the conditions that favor the stability of the polymorphisms such as the effects of mutation and genetic drift (15), natural and human perturbations (27,28), and genetic dominance in the host (33). In this paper, we extend Leonard's model (16,17) to allow for a multiple loci interaction between plants and their pathogens. A more realistic model for a multilocus gene-for-gene interaction is developed from Leonard's model that assumes single-locus inheritance in host resistance and parasite virulence. Our main objective is to investigate how a two-locus genetic system could affect the final outcome of coevolution in a gene-for-gene system.

Corresponding author: J. Segarra; E-mail address: segarra@pvcf.udl.es

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MATERIALS AND METHODS

We consider a parasite population causing disease in a wild plant species in a natural system. Both populations are assumed to be haploid and without recombination. Generations are discrete and non-overlapping. Pathogen spores land on the hosts at the beginning of each growing season and each host has one and only one spore landing on it. We consider that the host and pathogen populations are infinitely large and mingle randomly. The next genetic models account for gene frequency dynamics while ignoring changes in population size.

Model of the single-locus system. The simplest genetic model for gene-for-gene interactions assumes haploid single-locus inheritance in host resistance and parasite virulence similar to Jayakar's model (14). There are two alleles at the host locus, one for resistance (R) and one for susceptibility (S), and two alleles at the parasite locus, one for virulence (V) and one for avirulence (A). As in typical gene-for-gene interactions, the resistance only takes effect when the resistant host is attacked by an avirulent parasite. Otherwise, the host is susceptible.

In the model, the relative fitness of the avirulent pathogen on the susceptible plant is 1, $W_p(A,S) = 1$. The relative fitness of the avirulent pathogen on the resistant plant is $1 - t$, $W_p(A,R) = 1 - t$, in which t represents the effectiveness of resistance. The virulent pathogen always infects the host and has a relative fitness of $1 - k$, $W_p(V,S) = W_p(V,R) = 1 - k$, in which k is the cost of virulence. In the model, the relative fitness of the h th plant genotype when infected by the j th pathogen genotype is

$$W_H(j,h) = (1 - sW_{p(j,h)})(1 - c) \quad (1)$$

where s represents the disease severity and c is the cost of carrying a resistance allele. Table 1 indicates the fitness of the pathogen and host for each combination of their genotypes.

The frequencies of virulent and avirulent alleles in the pathogen population at the end of the i th host generation are n_i and m_i ($n_i + m_i = 1$), respectively. The frequencies of resistant and susceptible alleles in the plant population are p_i and q_i ($p_i + q_i = 1$), respectively. During the growing season, selection changes the virulence and resistance frequencies. The proportion of a gene in the next generation is the quotient between the fitness of the allele and

TABLE 1. Fitness matrices of the parasite and host in the single-locus gene-for-gene system^a

	Parasite		Host	
	Susceptible	Resistant	Susceptible	Resistant
Avirulent	1	$1 - t$	$1 - s$	$(1 - s(1 - t))(1 - c)$
Virulent	$1 - k$	$1 - k$	$1 - s(1 - k)$	$(1 - s(1 - k))(1 - c)$

^a t , effectiveness of resistance; s , severity of disease; k , cost of virulence; and c , cost of resistance.

TABLE 2. Fitness matrices of the parasite and host in the two-locus gene-for-gene system

Parasite genotypes ^a	Host genotypes ^b			
	S_1S_2	S_1R_2	R_1S_2	R_1R_2
Parasite fitness				
A_1A_2	1	$1 - t$	$1 - t$	$(1 - t)^2$
A_1V_2	$1 - k$	$1 - k$	$(1 - t)(1 - k)$	$(1 - t)(1 - k)$
V_1A_2	$1 - k$	$(1 - t)(1 - k)$	$1 - k$	$(1 - t)(1 - k)$
V_1V_2	$(1 - k)^2$	$(1 - k)^2$	$(1 - k)^2$	$(1 - k)^2$
Host fitness				
A_1A_2	$1 - s$	$(1 - s(1 - t))(1 - c)$	$(1 - s(1 - t))(1 - c)$	$(1 - s(1 - t)^2)(1 - c)^2$
A_1V_2	$1 - s(1 - k)$	$(1 - s(1 - k))(1 - c)$	$(1 - s(1 - t)(1 - k))(1 - c)$	$(1 - s(1 - t)(1 - k))(1 - c)^2$
V_1A_2	$1 - s(1 - k)$	$(1 - s(1 - t)(1 - k))(1 - c)$	$(1 - s(1 - k))(1 - c)$	$(1 - s(1 - t)(1 - k))(1 - c)^2$
V_1V_2	$1 - s(1 - k)^2$	$(1 - s(1 - k)^2)(1 - c)$	$(1 - s(1 - k)^2)(1 - c)$	$(1 - s(1 - k)^2)(1 - c)^2$

^a A , avirulence allele; V , virulence allele.

^b S , susceptible allele; R , resistance allele; t , effectiveness of resistance; s , severity of disease; k , cost of virulence; and c , cost of resistance.

population fitness. So, frequencies of the genes for virulence and resistance in the next growing season give

$$n_{i+1} = f(n_i, p_i) = \frac{n_i(1 - k)}{1 - p_i t(1 - n_i) - n_i k} \quad (2)$$

$$p_{i+1} = g(n_i, p_i) = \frac{p_i[1 - s(1 - t) - n_i s(t - k)](1 - c)}{(1 - s(1 - n_i k))(1 - c p_i) + s p_i(1 - n_i)(1 - c)}$$

This model differs from Leonard's model (16) on two points: (i) In the way in which the host's fitness is affected by the cost of carrying a resistance allele 1. They are now assumed to combine multiplicatively as in the model by Agrawal and Lively (1) rather than additively. This is probably a more realistic assumption for transferring the single-locus model directly to the multilocus model, especially for a large number of loci, n , and/or a high resistance cost, c (13). If the effect of various genes were combined additively, the fitness values of the host would be able to be less than zero. And, (ii) there is an immediate reciprocal feedback between the plant and pathogen populations.

Model of the two-locus system. The multilocus system can be extended by considering how each locus contributes to the overall reaction. According to the gene-for-gene hypothesis, host and pathogens interact at each of r loci, with a susceptible reaction occurring unless there is a match between a resistant host allele and an avirulent pathogen allele. It is assumed that the resistance effects at different loci are multiplicative and any other combinations of alleles at a locus (that is, $S-A$, $S-V$, $R-V$) do not contribute to the resistance reaction.

The relative fitness of the j th pathogen genotype is

$$W_p(j) = \sum_{h=1}^{n_H} W_{p(j,h)} p(h) \quad (3)$$

where $W_{p(j,h)}$ is the fitness of the j th pathogen genotype when it infects the h th plant genotype (as shown in Table 2), n_H is the number of plant genotypes (in our model $n_H = 4$), and $p(h)$ is the frequency of the h th plant genotype. The relative fitness of the h th plant genotype is

$$W_H(h) = \sum_{j=1}^{n_P} W_{H(j,h)} n(j) \quad (4)$$

where $W_{H(j,h)}$ is the fitness of the h th plant genotype when it infects the j th pathogen genotype (as shown in Table 2), n_P is the number of pathogen genotypes (in our model $n_P = 4$), and $n(j)$ is the frequency of the j th pathogen genotype. The virulence and resistance frequencies then change by selection as

$$n_{i+1}(j) = \frac{n_i(j)W_p(j)}{W_p} \quad (5)$$

$$p_{i+1}(h) = \frac{p_i(h)W_H(h)}{W_H}$$

where

$$\begin{aligned}\overline{W}_P &= \sum_{j=1}^{n_P} n(j) \sum_{h=1}^{n_H} W_P(j, h) p(h) \quad \text{and} \\ \overline{W}_H &= \sum_{h=1}^{n_H} p(h) \sum_{j=1}^{n_P} W_H(j, h) n(j)\end{aligned}\quad (6)$$

are the mean fitnesses of the pathogen and plant population, respectively.

Both models were also simulated with small recurrent mutations between alleles at each locus in both species. The effect of mutation at a rate, μ , is modeled as shown (15):

$$\begin{aligned}n' &= n + \mu(1 - n) - \mu n = n + \mu(1 - 2n) \\ p' &= p + \mu(1 - p) - \mu p = p + \mu(1 - 2p)\end{aligned}$$

in which n' and p' are the virulence and resistance frequencies after mutation occurs. For simplicity, the same μ is assumed in each direction in both species.

The simulations were carried out on a Pentium IV PC computer using the Mathematica version 4 software (Wolfram Research, Inc., Champaign, IL).

RESULTS

Single-locus system. The single-locus coevolutionary model 2 has four trivial EPs: (0, 0), (0, 1), (1, 0), and (1, 1). Moreover, it has a single internal EP (n_e, p_e) = $((-c + cs + st - cst)/(cks + st - cst), k/t)$. The stability of the steady states is determined by the magnitude of the eigenvalues λ_1 and λ_2 of the system. If either $|\lambda_1| > 1$ or $|\lambda_2| > 1$, then EPs are locally unstable since little perturbations from it grow with time (24). The mathematical conditions that determine the stability of these EPs are shown in Table 3. The evolution of the system towards monomorphism A-S (0, 0) requires the resistance cost, c , to be higher than the gain in fitness by the plant as a result of a lesser development of the disease. On

the other hand, the evolution of the system towards the EP A-R (0, 1) requires the virulence cost, k , to be higher than the effectiveness of resistance ($k > t$). Because neither of these conditions makes biological sense, all the EPs are unstable.

Computer simulation of the system shows the appearance of boom and bust cycles until the trajectories finally reach the point (1, 0). For example, for the values $t = s = 0.8$ and $k = c = 0.2$, the trajectory of the system finally tends towards the point (1, 0). However, the EP is unstable because the eigenvalues of the above-mentioned point are $\lambda_1 = 1.25$ and $\lambda_2 = 0.8$. The question is how the simulated trajectories can end up in the point (1, 0) if this is an unstable point. On simulating the system starting from an initial state very close to the point (1, 0), the trajectory moves away from the point, forms a cycle, and on passing near the point again, is trapped in it. The analysis of the numerical values shows how the frequency n reaches a value so close to 1 that, owing to the limited precision of the computer, the value of n is rounded up to 1. From then on, the value of $p > 0$ is obtained indefinitely.

Nevertheless, in the simulation of model 2, the values of the frequencies can approach 0 infinitesimally because the program maintains 16 significant digits and thus the value is different from 0. That is why it is proposed to represent the system through the evolution of the avirulence (m) and resistance (p) frequencies with the aim of avoiding the trajectories being trapped in the monomorphic point V-S. Thus, the single-locus system is represented with the following equations:

$$\begin{aligned}m_{i+1} &= f(m_i, p_i) = \frac{m_i(1 - pt)}{1 - m_i p_i t - (1 - m_i)k} \\ p_{i+1} &= g(m_i, p_i) = \frac{p_i[1 - s(1 - t) - (1 - m_i)s(t - k)](1 - c)}{(1 - s(1 - (1 - m_i)k))(1 - c p_i) + s t p_i m_i (1 - c)}\end{aligned}\quad (7)$$

Although the simulation of the system uses the avirulence frequency m , the results are presented using the virulence frequency n . As $n = 1 - m$ and 1 is an exact number of infinite precision, the value of n has at least 16 significant digits and thus is a value different from 1.

Obviously, model 7 is equivalent to model 2. Both models have the same EP and identical eigenvalues. Thus, the EPs are unstable. Simulating the single-locus system numerically with equation 7 shows that for any combination of parameters and initial condition, the system spirals outwards, approaching closer on each cycle to the outline of the phase space defined by the four trivial EPs (Fig. 1). Being a determinist model, the trajectories approaches infinitesimally to the outline. In reality, some of the alleles of the pathogen and host populations would end up dying out owing to the finite size of the population. Thus, the polymorphism observed is transitory.

The bi-directional mutation in the plant and in the pathogen modified the dynamics of the system qualitatively. Although the system has the same EPs, the dynamics depend on the value of the mutation rate. Very small mutation rate values, such as $\mu = 10^{-16}$, are enough for the system to escape from the outline of the space of phases and evolve towards a stable limit cycle. As mutation rate increases, the cycles around the interior EP become smaller and happen faster. Beyond a determined value of μ_c , a supercritical Hopf bifurcation (12) by which the interior EP becomes stable and the limit cycle disappears is obtained numerically. For values of $\mu > \mu_c$ the system evolves towards a stable interior EP (Fig. 1).

The above calculations were performed with reciprocal genetic feedback between plant and pathogen populations. In contrast, Leonard assumed a delayed feedback, so that $n_{i+1} = f(n_i, p_i)$ and $p_{i+1} = f(n_{i+1}, p_i)$. The change in frequency of resistance from one growing season to the next depends on the composition of the pathogen population at the end of the season. With this assump-

TABLE 3. Equilibrium points and stability of the single-locus gene-for-gene model^a

Equilibrium points	Eigenvalues	Stability condition ^b
(0, 0)	$\lambda_1 = 1 - k$ $\lambda_2 = \frac{(-1 + c)(1 - s + st)}{-1 + s}$	$c > \frac{st}{1 - s(1 - t)}$
(0, 1)	$\lambda_1 = \frac{-1 + k}{-1 + t}$ $\lambda_2 = \frac{-1 + s}{-1 + c + s - cs - st + cst}$	$k > t$ and $c < \frac{st}{1 - s(1 - t)}$
(1, 0)	$\lambda_1 = 1 - c$ $\lambda_2 = \frac{1}{1 - k}$	Unstable
(1, 1)	$\lambda_1 = \frac{1}{1 - c}$ $\lambda_2 = \frac{-1 + t}{-1 + k}$	Unstable
	$\left(\frac{-c + cs + st - cst}{cks + st - cst}, \frac{k}{t} \right)$ $\lambda_{1,2} = 1 \mp \sqrt{\frac{ck(t - k)(c - cs - st + cst)}{(1 - c)(1 - k)st^2}}$	Unstable

^a Described in Table 1 and equation 2.

^b Determined by the condition that the absolute values of both eigenvalues are less than 1.

tion, EPs were obtained. Similarly, the trivial EPs have the same λ eigenvalues as those obtained with reciprocal genetic feedback. However, different λ eigenvalues were obtained for the internal EP. It was shown that $|\lambda_{1,2}|=1$ for values of $c < 0.8$ (Appendix). By means of simulations, it was observed that the system generated closed cycles around the interior EP and that this EP is a center (Fig. 2). The cycle that describes the trajectory depends on the initial condition. The further the initial condition is from the interior EP, the bigger the cycle. Thus, the internal EP is neutrally stable. The systems with neutrally stable EP are structurally unstable, that is to say, small perturbations in the system produce a qualitative change in the system dynamic (12). In this sense, it was demonstrated numerically how the bi-directional mutation stabilizes the interior EP and destroys the cycles (Fig. 2). The higher the mutation rate, the greater the attraction towards the internal EP.

Two-locus system. The evolution of the system depends on the values of the parameters. For a wide range of low and medium values of the k and c parameters, the system forms cycles indefinitely (Fig. 3). This cyclical dynamic shows itself both in the evolution of the allelic frequencies of virulence and resistance and in the evolution of the genotypical frequencies of the pathogen and the plant. Similarly, it is seen how the complexity of the races fluctuates. The average number of virulence alleles per pathogen isolate is higher than the average number of resistance alleles per plant (Fig. 3). If the values of k and c associated with each locus are different, the coevolutionary trajectories are more complex. However, when the k and c values of the two loci are equal, after a transitory period, a regular cyclical pattern is observed. The effect of the mutation (μ) on the dynamic is similar to

its effect on the single-locus system. When there is no mutation, the cycles increase in size. With low mutation rates, stable cycles appear and when the mutation rate is high, ($\mu \approx 10^{-3}$ to 10^{-4}) the initial cycles tend toward an internal EP.

In the set of the area of the parameters, the two-locus system can also evolve towards monomorphic EPs (Fig. 4). The evolution towards the fixed point S_1S_2 - A_1A_2 requires the following. (i) The fitness of the A_1A_2 pathogen genotype must be superior to the fitness of any other genotype of the pathogen (A_1V_2 , V_1A_2 , V_1V_2) when it infects the S_1S_2 plant genotype, that is, $W_p(A_1A_2, S_1S_2) > W_p(A_1V_2, S_1S_2)$ or $W_p(V_1A_2, S_1S_2)$, from which we obtain $1 > 1 - k$, and $W_p(A_1A_2, S_1S_2) > W_p(V_1V_2, S_1S_2)$ gives $1 > (1 - k)^2$. Both conditions are met for $k > 0$. Thus, the evolutionary stability of the avirulent race A_1A_2 in the susceptible host occurs if these virulence genes are unnecessary. (ii) The fitness of the S_1S_2 host genotype must be higher than the fitness of any other genotype of the host (S_1R_2 , R_1S_2 , R_1R_2) when it infects the A_1A_2 pathogen genotype, that is, $W_H(A_1A_2, S_1S_2) > W_H(A_1A_2, S_1R_2)$ or $W_H(A_1A_2, R_1S_2)$, from where we obtain $1 - s > (1 - s(1 - t))(1 - c)$, and $W_p(A_1A_2, S_1S_2) > W_p(V_1V_2, S_1S_2)$ gives $1 - s > (1 - s(1 - t)^2)(1 - c)^2$.

Both conditions are met if

$$c > \frac{st}{1 - s(1 - t)} \quad (8)$$

If condition 8 is met, the resistance alleles become extinct as do the virulence alleles later.

In the same way that the condition of stability of the EP S_1S_2 - A_1A_2 from equation 8 is obtained, similar conditions are obtained for the other EP. The system evolves towards the fixed point made up of the possible genotypes of the plant with a single resistance

Fig. 1. Phase planes from simulations of the single-locus gene-for-gene model with reciprocal feedback. Parameter values of $t = 0.8$, $s = 0.8$, $k = 0.15$, $c = 0.03$, and number of generations = 3,000. Initial frequencies for virulence (n_0) and resistance (p_0) and mutation rate (μ) are varied. **A**, $n_0 = 0.95$, $p_0 = 0.2$, and $\mu = 0.0$. **B**, $n_0 = 0.95$, $p_0 = 0.2$, and $\mu = 10^{-7}$. **C**, $n_0 = 0.2$, $p_0 = 0.8$, and $\mu = 10^{-7}$. **D**, $n_0 = 0.2$, $p_0 = 0.8$, and $\mu = 10^{-4}$. Absolute values of the eigenvalues of the internal equilibrium point: **A**, $|\lambda_i| = 1.00213$; **B and C**, $|\lambda_i| = 1.00212$; and **D**, $|\lambda_i| = 0.9987$.

allele and the avirulent genotype of the pathogen $S_1R_2/R_1S_2-A_1A_2$ when

$$\frac{st(1-t)}{1-s(1-t)^2} < c < \frac{st}{1-s(1-t)} \quad (9a)$$

$$k > \frac{t}{2-t} \quad (9b)$$

Condition 9a prevents the invasion of the plant genotype with two resistant alleles, R_1R_2 , and the genotype of the susceptible plant S_1S_2 . Condition 9b establishes a minimum value of the virulence cost k so that the virulent pathogen races do not establish themselves. In that EP, both hosts S_1R_2 and R_1S_2 co-occur at the same proportion due to the assumptions on equal parameters values.

There is a small window in the parameter area for intermediate values of k and c in which the system evolves towards the EP $S_1R_2/R_1S_2-A_1V_2/V_1A_2$. The k and c values must be between

$$\frac{st(1-k)}{2(1-s(1-k)(1-t))} < c < \frac{st(1-k)}{st(1-k)+2(1-s(1-k))} \quad (10)$$

$$\frac{t}{2} < k < \frac{t}{2-t}$$

The simulations demonstrated that the evolution of the system towards any of the three above-mentioned EPs is independent of the initial virulence and resistance frequencies. Likewise, its global stability was confirmed numerically. The incorporation of the bi-directional mutation does not modify the system dynamic qualitatively towards the three EPs.

From the point of view of disease management, the existence of the stable EP $S_1R_2/R_1S_2-A_1A_2$ is very interesting. In this case, the

system evolves towards resistant crop population. For this reason, it is proposed to determine the stability condition for a system with r loci. Condition 9a, which prevents the invasion of other genotypes of the plant, is independent of the number of loci. However, condition 9b depends on the number of loci. To avoid a race i with one virulence allele ($A_1 \dots V_i \dots A_r$) from invading the avirulent population of the pathogen then

$$W_p(A_1 \dots A_i \dots A_r) > W_p(A_1 \dots V_i \dots A_r) \quad (11)$$

Let f be the frequency of the genotype of the plant with a single resistance allele complementary to the race i . Thus, the combined frequency of the remaining genotypes without that resistance allele will be $1-f$. From condition 11, it follows that

$$1-t > f(1-k) + (1-f)(1-k)(1-t) \quad (12)$$

or the equivalent form

$$f < \frac{k(1-t)}{t(1-k)} \quad (13)$$

In this EP, r genotypes of the plant with a single resistance gene co-exist. Thus, equation 13 is changed into

$$\frac{1}{r} < \frac{k(1-t)}{t(1-k)} \quad (14)$$

Finally, reordering the terms, we obtain

$$k > \frac{t}{t+r(1-t)} \quad (15)$$

Thus, in the gene-for-gene system with r loci, equations 9a and 15 define the region of the parameter area where the system

Fig. 2. Phase planes from simulations of the single-locus gene-for-gene model with delayed feedback. Parameter values of $t = 0.8$, $s = 0.8$, $k = 0.2$, $c = 0.1$, and number of generations = 3,000. Initial frequencies for virulence (n_0) and resistance (p_0) and mutation rate (μ) are varied. **A**, $n_0 = 0.85$, $p_0 = 0.20$, and $\mu = 0.0$. **B**, $n_0 = 0.20$, $p_0 = 0.80$, and $\mu = 0.0$. **C**, $n_0 = 0.20$, $p_0 = 0.80$, and $\mu = 10^{-6}$. **D**, $n_0 = 0.20$, $p_0 = 0.80$, and $\mu = 10^{-4}$. Absolute values of the eigenvalues of the internal equilibrium point: **A and B**, $|\lambda_{ci}| = 1$; **C**, $|\lambda_{ci}| = 0.999998$; and **D**, $|\lambda_{ci}| = 0.99998$.

evolves in the long term towards the stable EP of the avirulent pathogen race with r genotypes of the plant with a single resistance gene.

In coevolutionary systems with a large number of loci, other stable EPs are obtained, such as the avirulent pathogen EP and doubly-resistant plant. The multilocus system tends in the long term towards this EP independently of the initial condition when

$$\frac{st(1-t)^2}{1-s(1-t)^3} < c < \frac{st(1-t)}{1-s(1-t)^2} \quad (16a)$$

$$k > 1 - \frac{1-t}{\sqrt{1 - \left(1 - \frac{2}{r(r-1)}\right)t}} \quad (16b)$$

DISCUSSION

The observed polymorphism has traditionally been explained by a fitness cost of virulence in pathogens and a fitness cost of resistance in plants (6). A major assumption of the gene-for-gene population models is that both the resistance allele and the virulence allele have a negative pleiotrophic effect on fitness in the host and pathogen, respectively (16,31). In Leonard's model (16, 17), the fitness cost associated with resistance allele is additive. We assume that both the cost of each virulence allele and the cost of each resistance allele is multiplicative (1,25). With this assumption, the loss of fitness caused by a resistance or virulence allele is proportional to the fitness of the genotype. This means that the global loss of fitness of a genotype owing to having multiple resistance or virulence alleles always increases when the

Fig. 3. Simulation of pathogen and host genotypes dynamics from the two-locus coevolution model. Parameter values are $t = 0.8$, $s = 0.8$, $k = 0.15$, $c = 0.07$, and $\mu = 10^{-7}$. Initial frequencies for virulence (n_0) and resistance (p_0) are different in each locus. First locus: $n_0 = 0.8$ and $p_0 = 0.2$; second locus: $n_0 = 0.001$ and $p_0 = 0.001$. **A**, Frequencies of pathogen races A_1A_2 and V_1V_2 . **B**, Frequencies of host genotypes S_1S_2 and R_1R_2 . **C**, Frequencies of pathogen races A_1V_2 and V_1A_2 . **D**, Frequencies of host genotypes S_1R_2 and R_1S_2 . **E**, Average number of virulence alleles per pathogen isolate. **F**, Average number of resistance alleles per host plant.

number of these alleles increases given that the fitness of the genotype never reaches zero (except for total resistance, $t = 1$). Likewise, we assume that resistance at different loci acts multiplicatively.

The single-locus model has a slightly different internal EP than Leonard's model (16). With the reciprocal genetic feedback assumption, the internal EP is locally unstable and there is a smooth outward spiral toward the boundaries. In contrast, with the delayed feedback assumption the internal EP is neutrally stable (neither asymptotically stable nor unstable) and the solutions to the full model are always cycles, with the amplitude of the cycle determined solely by initial conditions (e.g., Jayakar's [14] and Barrett's [4] models). Although this cyclical behavior leads to the

coexistence of polymorphism in host and parasite (4,19), we argue that this model is a poor one for describing the observed polymorphisms. Because the model simply oscillates with whatever amplitude is determined by the initial conditions, and a small change in our assumptions might lead to large changes in the dynamics of the model. On the other hand, the polymorphisms would end up disappearing owing to the low frequency the alleles reach at some moment in the cycle. For the European barley mildew population, a population of 10^{19} conidia has been estimated during the month of maximum disease (32). However, in the simulations, frequencies below 10^{-19} are reached. The most probable is that the alleles of virulence and susceptibility are fixed given that the internal EP is close to the EP $n = 1$ and $p = 0$,

Fig. 4. Dynamics of pathogen races (A_1A_2 , A_1V_2 , V_1A_2 , and V_1V_2) and plant genotypes (S_1S_2 , S_1R_2 , R_1S_2 , and R_1R_2) in the two-locus coevolution model. Parameter values are varied: **A and B**, $t = 0.5$, $s = 0.5$, $k = 0.05$, and $c = 0.35$; **C and D**, $t = 0.5$, $s = 0.5$, $k = 0.35$, and $c = 0.2$; and **E and F**, $t = 0.5$, $s = 0.5$, $k = 0.3$, and $c = 0.11$. Initial frequencies for virulence, $n_0 = 0.2$, and resistance, $p_0 = 0.8$. Mutation rate, $\mu = 10^{-7}$.

especially if the values of the virulence cost and the resistance cost are very low (15).

Assuming reverse mutation for both host and pathogen in the single-model has an important effect on the system dynamics, similar to that in Leonard's model (15). In the delayed feedback model the internal EP is stabilized and approached in an oscillatory fashion by the addition of mutation. On the other hand, in the reciprocal feedback model, a low mutation rate produces stable limit cycles. And beyond a certain mutation rate, the limit cycle disappears and the internal EP stabilizes. The inclusion of the mutation reduces the probability of alleles disappearing because of their low frequency and favors the persistence of a balanced polymorphism (15).

In general, the multilocus model shows a cyclic and balanced polymorphism. More complex fluctuations are shown in the initial phase of the coevolutionary process when the initial allele frequencies of each locus are different. This final cyclic outcome is also described by Sasaki (25) in his five-locus gene-for-gene model. An important characteristic from the practical point of view of the Sasaki model (25) and ours is that for a wide region of the parameter space, the multilocus system evolves towards the genotype of the avirulent pathogen (A_1A_2) and the genotypes of the plant with one resistance allele (S_1R_2 and R_1S_2). Moreover, this EP is stable in both models. That means that the system evolves towards a state in which all the crop area is resistant to the pathogen population. The disease quantity that will develop depends on the effectiveness of the resistance and thus is equal to $1 - t$.

The theoretical and applied question in the management of disease is how to reduce the virulence of the pathogen or, from another point of view, how to increase the effectiveness of the use of the resistance. In fact, the agricultural situation where the farmer controls the fraction of resistance genes in the population is a specific case within the more general model where both the host and the pathogen populations evolve. Although the model presented describes a natural plant-pathogen system, its analysis supplies some strategic directives about how the race-specific genes could be used better for a long-lasting protection of the crops. This model gives us the following keys. (i) Use incomplete resistance ($t < 1$). If $t = 1$, the system cannot evolve towards avirulence. Moreover, the smaller t is, the easier it is to reach the stable coevolutionary outcome $S_1R_2/R_1S_2-A_1A_2$ as the pressure of selection on the virulence is lower. (ii) The virulence cost surpasses a certain threshold (equation 15) to prevent the invasion of the virulence alleles in the pathogen population. (iii) Mix various genotypes with different resistance genes. This allows the cost of the virulence threshold to be reduced so that the crop's resistance prevents the spread of a virulent race of the pathogen. We find that a random mixture of $r = 5$ single resistant plant genotypes requires a minimum virulence cost of $k = 0.45$ ($t = 0.8$) or 0.23 ($t = 0.6$) to prevent virulence invasion in the population. If, for example, the number of plant genotypes was $r = 10$, the virulence cost needed to prevent virulence invasion would be $k = 0.29$ ($t = 0.8$) or 0.13 ($t = 0.6$). Thus, it is theoretically possible to protect the host population by mixing a big enough number of resistant varieties. (iv) It is preferable to use cultivar mixtures and/or multiline cultivars than sow a pyramidal cultivar with multiple resistance genes. The same principle was previously obtained by Sasaki (25). On comparing condition 16b with condition 9b, it shows that to establish an avirulent population, the necessary virulence cost in a mixture of host genotypes each with various resistance alleles is higher than in a mixture of host genotypes with a single resistance allele. Of course, in the short term, a variety that is protected by multiple resistance genes unmatched by virulence genes is more likely to remain uninfected for longer than if these genes are introduced singly, simply because of the need for several mutations to occur together and the lower likelihood of this occurring. However, when the complementary mutating races

are already established in the population the pyramidal varieties are ineffective. In that situation, our model concludes that in a crop area sown by cultivar mixtures or multiline cultivars the virulence genes would be extinguished if the virulence cost exceeded a certain threshold. This threshold of 15 can be lowered by increasing the number of cultivars with a single resistance allele maintained in the crop population.

Other authors have also determined the virulence cost or the number of genotypes in the plant that have to be present in a mixture of varieties to prevent the dominance of complex races (13,20,21). However, these models only study the evolution of the pathogen population in a host population with a fixed size and genetic structure. A more realistic approximation would be to model the coevolution of the system together with the epidemiology of the disease (11). In one model, Barrett (3) showed that the level of plant-to-plant transmission has a profound effect on the evolutionary outcome. Probably, this and other epidemiological parameters have a decisive influence on the coevolution of the system and, therefore, on the threshold of the virulence cost needed to prevent the invasion of the virulence alleles in the pathogen population.

APPENDIX

Derivation of the stability of the internal EP in the single-locus gene-for-gene model with delayed feedback. The eigenvalues of the internal EP of the single-locus gene-for-gene model 2 with delayed genetic feedback assumption are

$$\lambda_{1,2} = a \mp \sqrt{a^2 - 1}$$

where

$$a = \frac{k(s(t-1)+1)(t-k)c^2 + st(k^2 + tk - 2t)c - 2(k-1)st^2}{2(c-1)(k-1)st^2} \quad (A1)$$

The local stability analysis of the EP depends on the a value

$$\begin{aligned} \lambda_1 &= a - \sqrt{a^2 - 1} \quad \text{if} \quad \begin{array}{ll} a > 1 & \text{then } 0 < \lambda_1 < 1 \\ a < -1 & \text{then } \lambda_1 < -1 \\ -1 \leq a \leq 1 & \text{then } \lambda_1 \text{ is a complex number and } |\lambda_1| = 1 \end{array} \\ \lambda_2 &= a + \sqrt{a^2 - 1} \quad \text{if} \quad \begin{array}{ll} a > 1 & \text{then } \lambda_2 > 1 \\ a < -1 & \text{then } -1 < \lambda_2 < 0 \\ -1 \leq a \leq 1 & \text{then } \lambda_2 \text{ is a complex number and } |\lambda_2| = 1 \end{array} \end{aligned}$$

In conclusion, if either $a > 1$ or $a < -1$ the internal EP is unstable. And if $-1 \leq a \leq 1$, the EP is neutrally stable.

Simplifying expression A1, we obtain

$$a = \frac{ck(k-t)(st-c(1-s+st))}{2(c-1)(k-1)st^2} + 1 \quad (A2)$$

The numerator is negative given that for the interior EP to exist it has to comply with

$$c < \frac{st}{1-s(1-t)} \quad \text{and} \quad k < t \quad (A3)$$

On the other hand, the denominator of A2 is positive. Thus, for any combination of parameters, then $a < 1$. In turn, to accept the hypothesis that the interior EP is neutrally stable then it must comply with $a > -1$,

$$\frac{ck(k-t)(st-c(1-s+st))}{2(c-1)(k-1)st^2} + 1 > -1 \quad (A4)$$

and from here, we obtain

$$\frac{k(t-k)}{1-k} < \frac{4(1-c)st^2}{c(st-c(1-s+st))} \quad (A4)$$

Condition A4 is very complex to deduce whether it is complied with or not. To obtain a simpler condition the following

simplification is applied. Given that

$$\frac{k(t-k)}{1-k} < \frac{k(1-k)}{1-k} < \frac{4(1-c)st^2}{c(st-c(1-s+st))} \quad (\text{A5})$$

a maximum figure is obtained for the value of k

$$k < \frac{4\left(\frac{1-c}{c}\right)st^2}{st-c(1-s+st)} \quad (\text{A6})$$

So that this makes biological sense, $k < t$ thus

$$1 < \frac{4\left(\frac{1-c}{c}\right)st}{st-c(1-s+st)} \quad (\text{A7})$$

If $4\left(\frac{1-c}{c}\right) > 1$, equation A7 is complied with. From this we obtain

$$c < 0.8 \quad (\text{A8})$$

In conclusion, a higher level has been obtained for the resistance cost that indicates that the interior EP of the single-locus gene-for-gene model with delayed feedback is neutrally stable. It has been demonstrated that this is valid for any combination of parameters whenever c is < 0.8 .

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