Polymorphism in multi-locus host-parasite co-evolutionary interactions

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ABSTRACT

Numerous loci in host organisms are involved in parasite recognition, such as MHC genes in vertebrates or genes involved in gene-for-gene (GFG) relationships in plants. Diversity is commonly observed at such loci, and at corresponding loci encoding antigenic molecules in parasites. Multi-locus theoretical models of host-parasite co-evolution predict that polymorphism is more likely than in single locus interactions because recurrent co-evolutionary cycles are sustained by indirect frequency-dependent selection as rare genotypes have a selective advantage. These cycles are stabilised by direct frequency-dependent selection, resulting from repeated re-infection of the same host by a parasite, a feature of most diseases. Here, it is shown that for realistically small costs of resistance and virulence, polycyclic disease and high auto-infection rates, stable polymorphism of all possible genotypes is obtained in parasite populations. Two types of epistatic interactions between loci tend to increase the parameter space in which stable polymorphism can occur with all possible host and parasite genotypes. In the parasite, the marginal cost of each additional virulence allele should increase, while in the host, the marginal cost of each addition resistance allele should decrease. It is therefore predicted that GFG polymorphism will be stable (and hence detectable) when there is partial complementation of avirulence genes in the parasite and of resistance genes in the host.
INTRODUCTION

Host-parasite interactions are recognized as a major evolutionary force producing biological diversity. Genetic variation for resistance reduces the probability that an individual parasite can infect an individual host (May and Anderson 1990) and conversely, genetic diversity at parasite recognition loci increases the range of potentially susceptible hosts. Spatial and temporal genetic polymorphism is commonly found in nature at loci involved in host-parasite recognition such as the Major Histocompatibility Complex (MHC) in vertebrates (Apansius et al. 1997; Hill 2001) or genes involved in gene-for-gene (GFG) relationships, a common feature of plant-parasite interactions (Laine 2004; Thrall et al. 2001). In both the MHC and GFG systems, hosts and parasites may have multiple interacting loci (Apansius et al. 1997; Hill 2001; Palomino et al. 2002). Interactions among several plant resistance (RES) genes and parasite avirulence (AVR) genes have been documented for numerous diseases, of which the best studied include barley powdery mildew (Jorgensen 1994), flax rust (Thrall et al. 2001) and rice blast (Dewit 1992), as well as several diseases of the model plant Arabidopsis thaliana (Holub 2001).

In multi-locus systems of host-parasite interactions, negative indirect frequency-dependence selection (FDS) is thought to account for the great polymorphism found in MHC genes (Apansius et al. 1997; Borghans et al. 2004; Hill 2001) and GFG genes (Frank 1993a; Salathe et al. 2005; Sasaki 2000; Segarra 2005). In this hypothesis, host and parasite genotypes have a selective advantage when they are rare in coevolving populations. This leads to sustained co-evolutionary cycles because when a parasite rare allele is selected, its frequency increases, selecting in turn for the corresponding resistant host genotype. It is hypothesised that these regular cycles of genotype frequencies prevent invasion by a single genotype, especially when mutations introduce new alleles in populations (Borghans et al. 2004; Sasaki 2000). An important question about co-
evolution is therefore whether or not multi-locus interactions are sufficient to maintain polymorphism by themselves, or if other ecological and biological factors are required.

In gene-for-gene (GFG) relationships in plants, resistance is induced if the plant has a resistance (\textit{RES}) gene enabling recognition of a specific parasite avirulence (\textit{AVR}) protein (DANGL and JONES 2001). The parasite is not detected by the host and resistance is not induced if the host has a susceptibility allele (\textit{res}) or the parasite has a virulence allele (\textit{avr}). The asymmetry of the GFG interaction implies that in the absence of other factors, there will be an ‘arms race’, as successive pairs of \textit{RES} and \textit{AVR} alleles are driven to fixation in host and parasite populations respectively (BERGELSON \textit{et al.} 2001; HOLUB 2001). Accounting for the diversity observed at host and parasite GFG loci (LAINE 2004; THRALL \textit{et al.} 2001) is a significant challenge because a universally virulent pathogen genotype with an \textit{avr} allele at each locus might be expected to become fixed as it can infect all plant genotypes (FRANK 1993a; SEGARRA 2005).

Conditions for the maintenance of polymorphism in GFG interactions have been studied in a single locus system, with a single matching pair of a host \textit{RES} gene and a parasite \textit{AVR} gene (TELLIER and BROWN 2007). Co-evolution implies the existence of indirect FDS, because the rate of natural selection on \textit{RES} depends on the frequency of \textit{avr} and \textit{vice-versa}. Polymorphism can be maintained only if there is also negative, direct FDS, such that the strength of natural selection for either the host resistance allele or the parasite virulence allele or both declines with increasing frequency of that allele itself (TELLIER and BROWN 2007). Thus, while costs of \textit{RES} and \textit{avr} are necessary to maintain polymorphism, they are not sufficient to do so in a single locus system (TELLIER and BROWN 2007) or in multi-locus GFG interactions (SASAKI 2000; SEGARRA 2005). In a single locus GFG interaction, direct FDS is generated if the parasite passes through more than one generation in the same host individual, a feature which is common to most plant diseases. Such polycyclic
diseases are characterized by an auto-infection rate, the percentage of parasite spores re-infesting the same host from one parasite generation to the next (Barrett 1980). In single locus GFG interactions, stable long-term polymorphism can be most readily maintained in host and parasite populations at high auto-infection rates (Tellier and Brown 2007).

With respect to the stability of polymorphism, multi-locus systems have similar behaviour as that of single locus GFG systems. For monocyclic diseases on annual plants (one parasite generation per host generation), long-term stable polymorphism with all possible host and parasite genotypes cannot be obtained (Sasaki 2000; Segarra 2005). However, multi-locus interactions and high mutation rates increase the variance of the lifetime of a mutation (Sasaki 2000; Segarra 2005). Note that the dynamics of genotype frequencies in multi-locus models is highly affected by stochastic processes (mutation, drift) when many loci are considered (Frank 1993a; Salathe et al. 2005; Sasaki 2000).

Here, we investigate whether the existence of multiple GFG loci further stabilises a system in which epidemiological and ecological factors generate direct FDS (Tellier and Brown 2007), or further increases the variance of the lifetime of transiently polymorphic alleles (Holub 2001; Salathe et al. 2005; Segarra 2005).

A key issue is to discover the epidemiological and genetic factors which cause polymorphism at host and parasite multiple loci to be transient or stable (arms race or trench warfare models) (Holub 2001; Stahl et al. 1999). We extend the results of Tellier and Brown (2007) to a multi-locus GFG system with polycyclic disease, assuming realistically small costs of RES and avr. Mutation is included in this model because stochastic processes (mutation and drift) have been shown to play an important role in multi-locus GFG co-evolution (Frank 1993a; Salathe et al. 2005; Sasaki 2000). We show that polymorphism can be maintained at several host and parasite loci when the auto-infection rate is high. Moreover, compared to a single locus GFG relationship, multi-
locus interactions diminish the minimum constitutive cost of each $RES$ and $avr$ allele necessary for polymorphism to be maintained.

A complication when moving from a single locus to a multi-locus model is the possible existence of interactions between loci. Functional studies of $avr$ or $RES$ alleles show increasing experimental evidence of epistatic effects between loci in parasites (BAI et al. 2000; KAY et al. 2005; MUDGETT 2005; WICHMANN and BERGELSON 2004). In microbial parasites of plants, AVR proteins have a dual role; as well as being triggers for induction of host defences upon recognition by RES proteins, some of them at least are pathogenicity effectors (ALFANO and COLLMER 2004; DANGL and JONES 2001; RIDOUT et al. 2006; SKAMNIOTI and RIDOUT 2005). There may be partial complementation between $AVR$ genes (BAI et al. 2000; KAY et al. 2005; MUDGETT 2005; SKAMNIOTI and RIDOUT 2005; WICHMANN and BERGELSON 2004), so increasing the number of mutations of $AVR$ genes to $avr$ alleles which have lost pathogenicity effector activity may have a synergistically negative effect on parasite fitness. In plants, on the other hand, $RES$ genes induce the expression of similar defence processes (BROWN 2003). Significant costs might therefore arise when one $RES$ gene is expressed (TIAN et al. 2003), but expression of numerous genes would not necessarily increase cost of defence very much (BERGELSON and PURRINGTON 1996). The models analysed here incorporate functions to describe epistasis between the costs of multiple $RES$ and $avr$ alleles, depending on their number. For example, the marginal cost of adding a single new $RES$ allele may decrease as the number of existing $RES$ alleles increases, while the marginal cost of adding an $avr$ allele may increase with the number of existing $avr$ alleles. These epistatic cost functions are shown here to increase the parameter space in which stable polymorphism can be maintained in both host and parasite. This supports the hypothesis that multi-locus GFG systems favour
the maintenance of polymorphism at individual loci with the assumption of realistically small costs of $RES$ and $avr$ alleles.

**TWO LOCUS GFG MODEL WITH MONOCYCLIC DISEASE (MODEL A)**

*The model:* Model A describes a GFG system for two interacting loci in the host and the parasite. Both organisms reproduce clonally. Two alleles, $RES$ and $res$ in the host and $AVR$ and $avr$ in the parasite, are present at each locus and are coded 1 and 0 respectively (FRANK 1993a; SASAKI 2000; THRALL and BURDON 2002). For example, a plant genotype with a $RES$ allele at the first locus and a $res$ allele at the second is described as $10$, as is the parasite genotype with $AVR$ at the first locus and $avr$ at the second. An incompatible interaction occurs when the host $RES$ allele matches the parasite $AVR$ allele at least at one of the two interacting loci (1 matching at one or more loci, FRANK 1993a). Following a common assumption of GFG relationships, an incompatible interaction results in the parasite being unable to infect the host successfully (Table 1, DANGL and JONES 2001). This model is based on TELLIER and BROWN (2007) and is a GFG system for monocyclic disease slightly simplified from SEGARRA (2005). Fitnesses of host and parasite genotypes are given in Table 1, with the following parameters: $s$ is the cost to a plant of being diseased, $u_1$ ($u_2$) is the cost of one (or two) $RES$ alleles, and $b_1$ ($b_2$) is the cost of one (or two) $avr$ alleles. For instance, in Table 1, the fitness of a $10$ parasite is 0 on $11$ and $10$ plant genotypes but $(1−b_1)$ on plant genotypes $00$ and $01$. Recurrence equations for the frequencies of the genotypes are given in the Appendix.

*Existence of equilibrium points:* Each genotype has an equilibrium frequency; for example, that of host genotype $11$ is defined as $\hat{RR} = RR_y = RR_{y+1}$. There are trivial
equilibria defined by fixation of one or two host or parasite genotypes. Host equilibria are thus fixation of double-\textit{RES} plants (\(RR=1\)), fixation of double-susceptibility (\(rr=1\)) and fixation of both single-resistant genotypes (\(Rr+rr=1\)). Similar conditions are found for parasite genotypes: \(AA=1\), \(aa=1\) and \(Aa+aA=1\). The conditions for stability of these trivial equilibria are given in Sasaki (2000) and Segarra (2005).

The main point of interest here is the existence of multi-locus polymorphism, defined as an equilibrium state with three or four host and parasite genotypes, as can be found in natural populations (Laine 2004; Thrall et al. 2001). This occurs at the non-trivial equilibrium, where host and parasite genotype frequencies are as follows (analysis of recurrence equations in Appendix with Mathematica 5.0, Wolfram Research 2003):

\[
\begin{align*}
&[1] \quad \frac{\hat{RR}}{RR} = \frac{b_2 + b_2 b_1 - 2 b_1}{1 - b_1} \quad \frac{\hat{R}}{R} = \frac{b_1 (1 - b_2)}{1 - b_1} \quad \frac{\hat{r}}{r} = 1 - b_2 \\
&[2] \quad \frac{\hat{AA}}{AA} = \frac{(1 - s)(2 u_1 - u_1 u_2 - u_2)}{s(1 - u_1)(1 - u_2)} \quad \frac{\hat{a}}{a} = \frac{(1 - s) (u_2 - u_1)}{s(1 - u_1)(1 - u_2)} \quad \frac{\hat{a}}{a} = \frac{s - u_2}{s(1 - u_2)}
\end{align*}
\]

Note that the equilibrium frequencies of host genotypes depend on costs of \(avr\) alleles (\(b_2\) and \(b_1\)) while parasite equilibrium frequencies are functions of the costs of \(RES\) alleles (\(u_2\) and \(u_1\)) and the cost of disease (\(s\)) (Frank 1992).

In Eq. [1] the conditions for all host genotypes to exist simultaneously are:

0 < \(\hat{r}r\) < 1 (this is always true because 0 < \(b_2 < 1\));

0 < \(\hat{r}R, \hat{R}r\) < 1 \(\Rightarrow b_2 < 1\) and \(b_1 < 1/(2-b_2)\), which is reasonable, as costs of virulence tend to be small (Bergelson and Purrington 1996; Brown 2003);

\[
[3] \quad 0 < \frac{\hat{RR}}{RR} < 1; \quad \frac{\hat{R}}{R} > 0 \Leftrightarrow b_2 > 2 b_1/(b_1 + 1)
\]

Previous GFG models have assumed multiplicative costs of two single \(avr\) alleles where \(b_2 = 1-(1-b_1)^2\) (Segarra 2005). Condition [3] is fulfilled if the cost of having two \(avr\)
alleles \((b_2)\) is greater than or equal to the multiplicative cost of two single \(avr\) alleles because \(1-(1-b_1)^2 > 2b_1/(b_1+1)\).

In Eq. \([2]\) the conditions for all parasite genotypes to exist simultaneously are:

0 < \(\widehat{aa} < 1\) \(\Rightarrow\) \(u_2 < s < 1\) otherwise virulent parasites are eliminated from the population.

0 < \(\widehat{Aa}, \widehat{aA} < 1\) \(\Rightarrow\) \(u_2 > u_1\), i.e. the cost of having two \(RES\) alleles must be larger than that of one \(RES\) allele (similarly \(\widehat{AA} > 0 \Rightarrow u_2 > u_1\)).

\[4\] \(0 < \widehat{AA} < 1; \widehat{AA} > 0 \Leftrightarrow u_2 < 2u_1/(u_1+1)\)

In previous GFG models, the costs of having two \(RES\) alleles have been multiplicative:

\(u_2 = 1-(1-u_1)^2\). Condition \([4]\) is not satisfied if the cost of having two \(RES\) alleles \((u_2)\) is equal to the multiplicative cost of two single \(RES\) alleles because \(1-(1-u_1)^2 > 2u_1/(u_1+1)\).

Eq. \([4]\) shows that when multiplicative costs are assumed (as in Salathe et al. 2005; Sasaki 2000; Segarra 2005), double-\(AVR\) parasites cannot be maintained in populations.

Epistasis of fitness costs of \(RES\) and \(avr\) alleles is thus essential for the existence of an interior equilibrium point with all four host and all four parasite genotypes.

**Stability of the equilibrium point:** Following analysis in Tellier and Brown (2007), we use a logit transformation of genotype frequencies in Model A (Appendix and ESM Section 1) which simplifies considerably the analysis of the genotype dynamics. At host generation \(g\):

\[5\]

\(f_{AA} = \log(Aa_g/aA_g); f_{aa} = \log(aa_g/AA_g); f_{rr} = \log(rr_g/RR_g)\) and \(f_{rr} = \log(rr_g/RR_g)\)

The change (\(\Delta\)) in the ratio of parasite genotype \(10\) and \(01\) frequencies between generation \(g\) and \(g+1\) is then:

\[6\]

\(\Delta f_{aa} = \log(Aa_{g+1}/aA_{g+1}) - \log(Aa_g/aA_g)\)
Thus the system of equations of model A (see Appendix, ESM Section 1) can be rewritten as:

$$\begin{pmatrix} \Delta f_{aa} \\ \Delta f_{uu} \\ \Delta f_{rr} \\ \Delta f_{rr} \end{pmatrix} = J_A \begin{pmatrix} f_{aa} \\ f_{uu} \\ f_{rr} \\ f_{rr} \end{pmatrix},$$

where $J_A$ is the jacobian matrix of the system.

The dynamics of the system is determined by analysis of the eigenvalues of $J_A$ (see Appendix). For a model with four variables, two pairs of eigenvalues ($\lambda_{1,2}$ and $\lambda_{3,4}$) are solutions of the characteristic polynomial equation of $J_A$. The pairs of eigenvalues can be real $\lambda_{1,2} = \alpha \pm \sqrt{\beta}$ (and $\lambda_{3,4} = \alpha \pm \sqrt{\beta}$) or complex $\lambda_{1,2} = \alpha \pm i\sqrt{\beta}$ (and $\lambda_{3,4} = \alpha \pm i\sqrt{\beta}$), with:

$$\alpha_i = \frac{\partial \Delta f_{aa}}{\partial f_{aa}} + \frac{\partial \Delta f_{rr}}{\partial f_{rr}} \quad \text{and} \quad \alpha_2 = \frac{\partial \Delta f_{uu}}{\partial f_{uu}} + \frac{\partial \Delta f_{rr}}{\partial f_{rr}} \quad \text{(see Appendix)}$$

An exact condition for stability of an interior equilibrium of this dynamical system with four variables (Eq. 7) is that the four eigenvalues of $J_A$ must lie within a unit circle centred on (-1,0) in the complex plane (KOT 2001; ROUGHGARDEN 1996). The following condition is derived from the Routh-Hurwitz criterion for stability of a dynamical system (KOT 2001; ROUGHGARDEN 1996):

$$-1 < \alpha_i < 0 \text{ and } -1 < \beta_i < 1 \text{ and } -1 < \alpha_j < 0 \text{ and } -1 < \beta_j < 1$$

For model A, the eigenvalues are:

$$\begin{cases} \lambda_{1,2} = \pm \sqrt{\beta} & \text{if } \beta < 0 \\ \lambda_{3,4} = \pm \sqrt{\beta} & \text{if } \beta > 0 \end{cases}$$

with $\beta_i = \frac{(b_i - 2b_i(1-b_i))(u_i-u_i(2-u_i))(s-u_i)}{s(1-b_i)(1-u_i)(1-u_i)}$ and $\beta_j = \frac{(1-s)(1-b_j)b_i^2(u_i-u_j)^2}{s(1-b_i)(1-u_i)}$.

Consequently, there is always at least one eigenvalue which does not verify condition [9], and the interior, non-trivial equilibrium (Eqs. [1,2]) is always unstable. The mathematical reason for this is that all diagonal elements of $J_A$ are zero, therefore $\alpha_i = \alpha_2 = 0$ (Eq. [8], Appendix and ESM Section 1). The elements of $J_A$ are the rates of natural selection on the
ratio of genotype frequencies. For example, $\partial \Delta f_{aa} / \partial f_{aa}$ is the rate of selection on the double-avr parasite genotype (00) as a function of its own frequency, i.e. the rate of direct FDS on 00 parasites (TELLIER and BROWN 2007). Therefore, $\alpha_1$ and $\alpha_2$ are the sums of the direct FDS coefficients for the four ratios of genotype frequency (Eq. [8]). Eq. [10] demonstrates that for monocyclic diseases, there is no direct negative FDS for host or parasite genotypes, and a polymorphic state with three or four host and parasite genotypes is always unstable.

**TWO LOCUS GFG MODEL WITH POLYCYCLIC DISEASE**

Model description: Model B is a multi-locus GFG system with polycyclic disease, where polycyclic pathogens undergo several ($G$) multiplicative generations during one host generation. Here, the simplest case of $G=2$ parasite generations per host generation is considered. The autoinfection rate ($\psi$) is the percentage of infectious spores that re-infect the same host plant in the second parasite generation (BARRETT 1980; TELLIER and BROWN 2007). The cost to a plant of being diseased increases with the number of successive parasite infections, with a maximum fitness loss of $\phi$ after $G$ parasite generations (CAMPBELL and MADDEN 1990; TELLIER and BROWN 2007). The loss of plant reproductive output caused by disease increases disproportionately with $\pi$, the number of successful parasite generations on a host plant ($\pi \leq G$) because, as the parasite grows multiplicatively, corresponding damage is done to the host (CAMPBELL and MADDEN 1990). The plant fitness ($F$) is a decreasing function of $\pi$ where $z$ is a parameter defining the shape of the disease curve ($z>1$) (TELLIER and BROWN 2007).

$$F = 1 - \phi(\pi / G)^z$$
\( \varepsilon \) is the decrease of plant fitness after \( \pi = 1 \) infection (\( \varepsilon = \phi(1/2)^{\pi} \): Eq. 11, \( G=2, \pi =1 \)). For simplicity, the parasite reproductive fitness does not depend on \( \pi \). Deterministic equations for evolution of genotype frequencies in time are given below and can be obtained from fitnesses given in Tables S1 and S2 (ESM, Section 2). Table 2 is a summary of equations in Model B with only auto-infection (\( \psi=1 \)).

As an example, the outcome of infection on plant genotype \( 11 \) is described below (Table S1 in ESM, Section 2). In the first parasite generation (\( \pi=1 \)), a \( 11 \) plant can encounter parasite genotypes \( 11, 01 \) or \( 11 \) which cannot infect it successfully. In the second parasite generation (\( \pi=2 \)), that plant can then either \( i) \) encounter spores from of the same genotypes (frequencies \( AA_1, Aa_1, aA_1 \)) which cannot infect it (the plant’s fitness is then \( 1-u_2 \)) or \( ii) \) be infected by the super-virulent genotype \( 00 \) (frequency \( aa_1 \)), so its fitness is \( (1-u_2)(1-\varepsilon) \).

On the other hand, when \( 11 \) plants are infected by a super-virulent \( (00) \) parasite at \( \pi=1 \) (frequency \( aa_g \) at the start of generation \( g \)) the following occurs at \( \pi=2 \):

\( i) \) A proportion \( \psi \) of these plants remain infected by the same parasite genotype (auto-infection). A proportion \( \psi aa_g RR_g \) of all the plants in the population has fitness \( (1-u_2)(1-\phi) \) after two consecutive successful infections.

\( ii) \) A proportion \( 1-\psi \) are allo-infected by virulent parasites (here, only those with the super-virulent genotype \( 00 \) ) produced in the first parasite generation with frequency \( aa_1 \) (proportion \( (1-\psi) aa_g aa_1 RR_g \)). These plants are also infected twice and have fitness \( (1-u_2)(1-\phi) \).

\( iii) \) A proportion \( 1-\psi \) may encounter spores from the first parasite generation of the genotypes \( 01, 01 \) or \( 11 \) (frequencies \( AA_1, Aa_1 \) and \( aA_1 \)) which cannot infect. Their fitness is \( (1-u_2)(1-\varepsilon) \).
Formulae: The following are deterministic equations for a two-locus GFG system with two parasite generations per host generation with independent fitness costs of host resistance or parasite virulence alleles at different loci and no mutation. Frequencies of parasite genotypes after the first parasite generation are identical to those in Model A (see Table 2 and ESM, section 1):

\[
\frac{aa_g}{AA_g} = \frac{aa_g(1-b_g)}{AA_g \psi + rR_g}
\]

\[
\frac{Aa_g}{aA_g} = \frac{Aa_g(1-b_g)(rr_g + rR_g)}{aA_g(1-b_g)(rr_g + Rr_g)}
\]

After the second parasite generation (i.e. at the start of the next host generation, \(g+1\)), parasite genotype frequencies are:

Ratio of parasite genotype frequencies 00 to 11:

\[
\frac{aa_{g+1}}{AA_{g+1}} = \frac{RR_g[aa_g(1-aa_g) + aa_g \psi(1-b_g) + aa_g(1-\psi)aa_g] + rr_g[aa_g \psi(1-b_g) + aa_g(1-\psi)]}{(1-b_g) + rR_g[aa_g(1-aa_g) + aa_g \psi(1-b_g) + aa_g(1-\psi)] + rR_g[aa_g(1-aa_g) + aa_g \psi(1-b_g) + aa_g(1-\psi)]}
\]

Ratio of parasite genotype frequencies 10 to 01:

\[
\frac{Aa_{g+1}}{aA_{g+1}} = \frac{rr_g[Aa_g(1-aa_g) + aa_g \psi(1-b_g) + aa_g(1-\psi)] + rR_g[aa_g(1-aa_g) + aa_g \psi(1-b_g) + aa_g(1-\psi)]}{rr_g[Aa_g(1-aa_g) + aa_g \psi(1-b_g) + aa_g(1-\psi)] + rR_g[aa_g(1-aa_g) + aa_g \psi(1-b_g) + aa_g(1-\psi)]}
\]

The ratios of host genotype frequencies at the end of host generation \(g\) and the start of generation \(g+1\) are:

Ratio of host genotype frequencies 01 to 10:
Ratio of host genotype frequencies 00 to 11:

$$\frac{rr_{g+1}}{RR_{g+1}} = \frac{rr'_{g}(1-\phi)}{RR'_{g}(1-u_{r})}$$

**Existence of equilibrium point (ψ=1):** The same trivial equilibrium points exist as in Model A. The complexity of the equations in Model B constrains analysis of the non-trivial equilibrium point to the case when there is only auto-infection (ψ=1). Owing to quadratic terms and the non-linear behaviour of Model B, the following assumptions were made: i) $\tilde{aa} = a\tilde{a}(1-b_{1})/(1-b_{2}\tilde{a}a)$ and, ii) $b_{1}$ is small so that $(1-b_{1})\approx1$ and $(1-b_{1})(1-b_{2})\approx(1-b_{2})$. The accuracy of these approximations and of the following equilibrium frequencies was tested numerically across a wide range of parameter values. Theoretical values obtained with Mathematica 5.0 (WOLFRAM RESEARCH 2003) were compared to numerically simulated values, calculated as the mean of genotype frequencies over the last 100 generations of 5,000 simulated host generations. These approximations (Eqs. 12,14) are accurate for moderate to high values of $\phi$, but less accurate for values of $\phi<\phi_{u_{1}}$ or values of $\tilde{AA}$ when $u_{1}<\phi<\phi_{u_{2}}$ (ESM, Section 3).

$$\tilde{aa} = \frac{\omega-\sqrt{\omega^{2}-4\varepsilon(\phi-u_{2})}}{2(1-u_{2})(\varepsilon+b_{2}(\phi-\varepsilon))}$$

$$\tilde{aA} = \tilde{Aa} = \frac{\omega-\sqrt{\omega^{2}-4(\phi-u_{1})(\phi-u_{2})(1+\phi)}}{2(1-u_{1})(1-u_{2})(\varepsilon+b_{2}(\phi-\varepsilon))}$$
\[ AA = \frac{\omega - \sqrt{\omega^2 - 4\epsilon(1 + \phi)(2u_1 + u_2 u_2 - u_2)}}{2(1 - u_1)(1 - u_2)(\epsilon + b_2(\phi - \epsilon))} \]

where \( \omega = (1 - b_2)(\phi + \epsilon) + b_2(\phi - \epsilon - u_2(1 - \epsilon)) \)

In Eq. [12] the conditions for all parasite genotypes to exist simultaneously are:
\[ \hat{a}a > 0 \iff \phi > u_2 \text{ and } \hat{A}a > 0 \iff u_2 > u_1 \text{ (similarly for } \hat{a}A > 0 \text{).} \]

[13] \[ \hat{A}A > 0 \iff u_2 < 2u_1/(1 - u_1) \]

In contrast to Model A (Eq. [4]), condition [13] is fulfilled if the cost of having two RES alleles \( (u_2) \) is lower than or equal to the multiplicative cost of two single RES alleles. The equilibrium frequency of double-AVR parasites increases when the difference between \( u_2 \) and \( u_1 \) diminishes.

[14] \[ \hat{r}r = \frac{(3 - \hat{a}a - 2b_2\hat{a}a)(1 - b_2)^2}{3 - 2a a - 2b_2} \]
\[ \hat{r}R = \hat{R}r = \frac{b_2(3 - \hat{a}a)(1 - b_2)^2}{(1 - b_2)(3 - 2a a - 2b_2)} \]
\[ \hat{R}R = \frac{(b_2 - 2b_1 + b_2 b_2)(1 - b_2)^2}{3 - 2a a - 2b_2} \]

In Eq. [14] the conditions for all host genotypes to exist simultaneously are:
\[ \hat{r}r > 0 ; \hat{r}R > 0 ; \hat{R}r > 0 \text{ because } 3 > \hat{a}a + 2b_2\hat{a}a \text{ and } 3 > \hat{a}a + 2b_2 \text{ by definition.} \]

[15] \[ \hat{R}R > 0 \iff b_2 > 2b_1/(1 + b_1) \]

Condition [15] is identical to eq. [3], and is fulfilled if the cost of having two avr alleles \( (b_2) \) is greater than or equal to the multiplicative cost of two single avr alleles. The equilibrium frequency of double-RES plants becomes higher with a greater difference between \( b_2 \) and \( b_1 \).
**Stability of the equilibrium point:** The local stability of the non-trivial equilibrium is analysed when there is only auto-infection ($\psi=1$) because in this situation, stable polymorphism occurs over a wider parameter space (TELLIER and BROWN 2007). The main differences from Model A are that the following coefficients are not zero (ESM, Section 4):

\[
\frac{\partial \Delta}{\partial f_{aa}} = \frac{-Rr_g AA_g}{1-b_2 aa_g + Rr_g (AA_g + Aa_g)} = x_5
\]

This coefficient is always negative as $1+Rr_g (AA_g + Aa_g) > -b_2 aa_g$

\[
\frac{\partial \Delta}{\partial f_{aa}} = \frac{-(1-rr_g)(1-b_2)aa_g AA_g}{(1-b_2 aa_g)(2-2b_2 aa_g + (1-rr_g)(1-aa_g))} = x_6
\]

This coefficient is also negative as $2+(1-rr_g)(1-aa_g) > 2b_2 aa_g$

The Jacobian matrix for Model B, $J_B$, can thus be rewritten:

\[
J_B = \begin{pmatrix}
x_5 & 0 & x_3 & 0 \\
0 & x_6 & 0 & x_4 \\
x_1 & 0 & 0 & 0 \\
0 & x_2 & 0 & 0
\end{pmatrix}
\]

Approximations for the elements $x_1, x_2, x_3, x_4$ are derived in the ESM (Section 4). $J_B$ is diagonalizable and has two pairs of eigenvalues ($\lambda_{1,2}$ and $\lambda_{3,4}$) which can be real:

\[
\lambda_{1,2} = \frac{1}{2}\left(x_5 \pm \sqrt{x_5^2 + 4x_1 x_3}\right) \text{ if } x_5^2 + 4x_1 x_3 > 0
\]

and

\[
\lambda_{3,4} = \frac{1}{2}\left(x_6 \pm \sqrt{x_6^2 + 4x_2 x_4}\right) \text{ if } x_6^2 + 4x_2 x_4 > 0
\]

or complex:

\[
\lambda_{7,2} = \frac{1}{2}\left(x_5 \pm i\sqrt{-x_5^2 - 4x_1 x_3}\right) \text{ if } x_5^2 + 4x_1 x_3 < 0
\]

and

\[
\lambda_{8,4} = \frac{1}{2}\left(x_6 \pm i\sqrt{-x_6^2 - 4x_2 x_4}\right) \text{ if } x_6^2 + 4x_2 x_4 < 0
\]
A necessary condition for stability (Eq. [9]) is verified because $x_5 = \frac{\partial \Delta f_{aa}}{\partial f_{aa}}$ and $x_6 = \frac{\partial \Delta f_{aa}}{\partial f_{aa}}$ are both negative. However, a second condition for stability is that both absolute values of the discriminants of the characteristic polynomial, $|x_5^2 + 4x_1x_5|$ and $|x_6^2 + 4x_2x_4|$, must be less than 1 (Eq. [9]). Analytical derivation of this second condition is not possible because of the non-linearity of equations in Model B, and because only approximations of the equilibrium genotype frequencies can be obtained.

**Simulation methods:** Host and parasite genotype frequencies were therefore simulated numerically for different values of $\psi$ and $\phi$. Simulations were run in Matlab version 7.0 (Release 14) for 15,000 host generations, by which time stable behaviour (or genotype fixation) was achieved, with different sets of initial host and genotype frequencies (all host and parasite genotypes were present at the beginning of each simulation). The system was considered to be stable when the amplitude of the fluctuations of each genotype frequency decreased in time and converged towards an equilibrium value for any of the initial allele frequencies tested.

Mutations, especially with high mutation rates, regularly introduce new rare genotypes into host and parasite populations (Kirby and Burdon 1997; Salathe et al. 2005; Sasaki 2000). We therefore compared results of simulations with and without mutation. One set of simulations was done with a mutation rate of $10^{-5}$. A second set of simulations assumed two different mutation rates: $10^{-5}$ if a mutation results in a loss of function (from RES to res and from AVR to avr), and $10^{-8}$ for a gain of function mutation in the reverse direction (from res to RES or from avr to AVR) (Kirby and Burdon 1997). A host or parasite genotype was considered lost from a population when its frequency was lower than $10^{-6}$, but could be subsequently re-introduced by mutation (if any). On the other
hand, a genotype was fixed in a population when its frequency was higher than $1 \times 10^{-6}$. For a given set of parameter values, the results of the different types of simulations (with and without mutations, or with different initial genotype frequencies) were compared. The description of results follows with Models B1 and B2.

**MULTIPLICATIVE CONSTITUTIVE COSTS (MODEL B1)**

Model B1 is a two locus GFG system with multiplicative costs of $RES$ and $avr$ alleles, *i.e.* no epistatic interactions among loci for fitness values (Frank 1993b; Salathe et al. 2005; Sasaki 2000; Segarra 2005). Recurrence equations for genotype frequencies are those of Model B, where $b_2$ and $u_2$ are the costs of having two $RES$ or $avr$ alleles.

\[
\begin{align*}
&b_2 = 1 - (1 - b_1)^2 \quad \text{and} \quad u_2 = 1 - (1 - u_1)^2
\end{align*}
\]

Simulations were run with $b_1 = u_1 = 5\%$ and $b_2 = u_2 = 9.75\%$, these values being chosen to allow comparison with single locus results (Tellier and Brown 2007). When there is only auto-infection ($\psi = 1$), the double-$RES$ genotype has a very low equilibrium frequency ($<10^{-4}$, Eq. [14]). Model B1 is tested numerically to determine if the equilibrium point with all host and parasite genotypes exists for different values of $\psi$ and to discover the range of parameter values of $\psi$ and $\phi$ for which the equilibrium point is stable.

**Results:** Results of simulations are summarized in Figure 1 by the state of the system (stable or unstable) and the genotypes maintained in the host (Figure 1a) and parasite (Figure 1b) populations. Figure 2a shows the dynamics of host genotype frequencies in one simulation typical of Area B of Figure 1a with stable polymorphism of three host genotypes ($00, 10, 01$). Similarly, Figure 2b shows the dynamics of parasite gene frequencies in Area I of Figure 1b where there is stable polymorphism of all four parasite
genotypes, with genotype 11 at a very low frequency. Typical simulation results for each area of Figure 1 are provided in the ESM (Section 4).

If disease severity is smaller than the cost of one RES allele, there is fixation of host res alleles (00) because there is no net advantage to resistance (Area A in Figure 1a). As a result, AVR alleles (11) are fixed in the parasite population because virulence is costly (Area G in Figure 1b). In mathematical terms, Areas A and G correspond to the situation with zero (or negative) equilibrium frequencies of parasite genotypes 01, 10 and 11 because $\phi<u_1<u_2$ (Eqs. [12]). The limit of Areas A and G is thus the cost of one RES allele ($\phi=u_1$).

At medium to high autoinfection ($\psi$) and low to medium disease severity ($\phi$), there is stable polymorphism of three host genotypes (00, 10, 01) (Figure 1a, Area B and Figure 2a). Double resistant plants (11) are eliminated from the population because the benefit of being super-resistant (not being infected) is not large enough to overcome the cost of having two RES alleles. As a consequence, when $u_1<\phi<u_2$ and autoinfection rates are intermediate to high, because avr alleles are costly, double-avr parasites are eliminated from the parasite population (Area H in Figure 1b). Stable polymorphism with parasite genotypes 11, 10 and 01 occurs. Mathematically, Area H corresponds to a situation where the double-avr equilibrium frequency is zero (or negative) as $\phi<u_2$ (Eq. [12]).

With increasing $\phi$ and high auto-infection rates, all four parasite genotypes (00, 01, 10, 11) coexist in stable polymorphism (Area I, Figure 1b) because there is direct FDS acting on parasite genes (Eqs. [16, 17]), and the interior equilibrium for all possible parasite genotypes exists (Eq. [12]; Figure 2b). The parameter space in which polymorphism is stable diminishes with increasing $\phi$, because resistant genotypes (10 and 01) are selected more strongly, in turn selecting for double-avr parasites (00). Therefore, at intermediate to high $\psi$, increasing $\phi$ favours double-avr parasites and counter-selects double-AVR
genotypes (11) (Area J). In Area J, values of $\psi$ and $\phi$ do not allow the existence of an interior equilibrium frequency for the double-$AVR$ parasite (eq. [12]).

A key result is that the size of Areas H, I, and J in Figure 1b matches that of Area B in Figure 1a. This is because stability of polymorphic state depends on the strength of direct FDS against strength of indirect FDS, both of which being determined by $\psi$ and $\phi$. Therefore, conditions for stability are identical for host and parasite populations, as shown for single-locus interactions (Tellier and Brown 2007), and only the existence of equilibrium frequencies of the various genotypes discriminates between the different dynamics in host and parasite populations. Moreover, the equilibrium frequency of the double-$avr$ genotype (00) increases with $\phi$ (Eq. [12]) and always has the highest frequency in the parasite population. This is in agreement with observations from surveys in natural populations (Bevan et al. 1993; Dinoor and Eshed 1987; Thrall et al. 2001; Figure 3b).

When there is a high cost of disease (high $\phi$) (Figure 1a,b), there is first strong selection for resistant host genotypes 01, 10, 11. They selects strongly for virulent parasite genotypes, and especially the double-$avr$ genotype (00). Very high frequencies of double-$avr$ parasite (Area K, Figure 1b) then lead to long-term increase of the double-susceptible genotype frequency (00) because RES alleles are costly. At very high $\phi$, this results in the fixation of the double-$avr$ parasite genotype and the double-susceptible host genotype (Areas E and L in Figure 1). The dynamical system is unstable when $\phi$ increases, because the indirect FDS over-rides the stabilising effect of direct frequency-dependent stabilizing effect (Areas D and K).

Realistic mutation rates do not affect the behaviour of the model (stability or unstability) or frequencies at equilibrium in stable areas (B in Figure 1a and H, I and J in Figure 1b). Without mutation, when the system is unstable (Areas D, E in Figure 1a and K,
L in Figure 1b) there is fixation of the double-avr parasite genotype. Mutations can sustain stochastic co-evolutionary cycles by recurrent introduction of new rare genotypes in Areas D and K, following an arms race model. However, as the system has unstable behaviour (Areas D, E in Figure 1a and K, L in Figure 1b) each co-evolutionary cycle results in the fixation of the double-avr parasite until a new mutation arises.

**EPISTATIC INTERACTIONS AMONG LOCI (MODEL B2)**

In Model B2 epistatic interactions are assumed between loci in both host and parasite. General expressions are shown here for the costs of multiple avr (or RES) alleles in a multilocus GFG system with n loci. The maximum cost of having n avr (or RES) alleles is $b_{\text{max}} (u_{\text{max}})$.

The cost $b_k$ of having $k$ avr alleles is thus:

$$b_k = b_{\text{max}} \left(\frac{k}{n}\right)^\theta \text{ with } 0 \leq k \leq n$$

The marginal cost of each additional mutation from AVR to avr increases exponentially with the number of existing avr alleles, such that the loss of two AVR functions is more costly to the pathogen than expected if the costs were independent, therefore we choose $\theta > 1$, and the cost curve has a convex shape. In Model B2, $n=2$ so $b_{\text{max}} = b_2$.

On the other hand, the marginal cost of each additional RES allele diminishes with increasing number of existing RES alleles, so the cost of two RES alleles is lower than expected if the costs at different loci were independent. The cost curve has a concave shape when $\xi < 1$. The cost $u_k$ of having $k$ RES alleles is thus:

$$u_k = u_{\text{max}} \left(\frac{k}{n}\right)^\xi \text{ with } 0 \leq k \leq n$$

In Model B2, $n=2$ so $u_{\text{max}} = u_2$. In order to compare results from Models B1 and B2, the cost of having two alleles is fixed to 0.0975 ($u_2 = b_2 = 0.0975$, Eq. [6]). The only difference
between Models B1 and B2 is then the cost of having one allele with $u_1=0.072$ ($\xi=0.4$) and $b_1=0.024$ ($\theta=2$). Results for each area of Figure 3 can be seen in ESM, section 6.

**Results:** In Model B2, stable polymorphism with all four genotypes is maintained in host (Area C in Figure 3a and Figure 4a) and parasite populations (Area I in Figure 3b and Figure 4b) with medium to high $\psi$. This occurs because the equilibrium frequency of double-RES hosts increases in proportion to the difference between $b_1$ and $b_2$ (Eq. [14]). In other words, increasing the cost of having two avr alleles compared to the cost of one avr allele favours parasite genotypes 01 and 10, thus enhancing selection for double-RES host genotypes, and increasing the value of $m_{RR}$ (Figure 4a). In simulations conducted with epistasis between parasite loci and multiplicative costs of RES alleles, there is also stable polymorphism with all four host and parasite genotypes (data not shown). Moreover, epistasis among host loci which decreases the difference between $u_1$ and $u_2$, has the effect of diminishing the equilibrium frequency of double-avr parasites, and increasing that of double-AVR parasites. The diminution of the cost of having two RES alleles compared to that of one RES allele decreases selection for host genotypes 01 and 10, thus enhancing selection for double-AVR genotypes, and increasing the value of $m_{AA}$ (Figure 4b).

The total stability area for the host in Model B2 (C and B in Figure 3a) has a comparable size but is not identical to Area B in Figure 1a (Model B1). For the parasite, stability Areas H and I together in Figure 3b have a comparable size but are not identical to Areas H, I and J together in Figure 1b (Model B1). The areas for stable polymorphism are comparable because conditions for stability depend on the strength of direct FDS, which mainly depends on $\psi$ and $\phi$ (see Eq. [19-20]). However, stability conditions also depend on costs of RES and avr alleles (Eq. [16, 17]), which differ between Model B1 and B2, which is why stability areas do not overlap exactly between Figures 1 and 2. Although all four
host and parasite genotypes can be maintained, double-res plants and double-avr parasites have higher equilibrium frequencies than the other genotypes, in agreement with results from natural populations (Thrall et al. 2001; Figure 4 a and b).

In Model B2, the equilibrium point with the four host and parasite genotypes exists. However, when $\phi$ increases, because indirect FDS overrides the stabilising effect of direct FDS, this equilibrium state becomes unstable in Areas F (Figure 3a) and Area M (Figure 3b).

Other results from Model B2 are similar to Model B1. If $\phi < u_1$, host genotype 00 (Area A in Figure 3a) and parasite genotype 11 (Area G in Figure 3b) become fixed. When $u_1 < \phi < u_2$, and $\psi$ is intermediate to high, double-RES genotypes and double-avr parasites are eliminated respectively from the host (Area B in Figure 3a) and parasite (Area H in Figure 3b) populations. In Areas B and H, there is stable polymorphism with three host and three parasite genotypes. Finally, at very high $\phi$, there is fixation of the 00 host and parasite genotypes (Areas E and L in Figure 3). Simulations for more than two loci generalize our conclusions from Models B showing the generality of the approach ($n=3$ in ESM, Section 7).
DISCUSSION

As in the single-locus model of Tellier and Brown (2007), polycyclic disease gives rise to conditions which stabilise polymorphism at equilibrium (Model B, c.f. Model A). Stable polymorphism with three or four host genotypes and four parasite genotypes occurs at intermediate to high rates of auto-infection (Model B). For monocyclic disease (Model A), there is no direct FDS in the host \( \partial \Delta f_{rr} / \partial f_{rr} = \partial \Delta f_{rr} / \partial f_{rr} = 0 \) or parasite \( \partial \Delta f_{aa} / \partial f_{aa} = \partial \Delta f_{aa} / \partial f_{aa} = 0 \) populations (eq. \[10\]). Polymorphism is stabilized in polycyclic diseases, because the stability of GFG systems depends on the outcome of infection in the first parasite generation \( (g,1) \) influencing the second parasite generation \( (g,2) \). Model B extends the principle of single-locus GFG co-evolution (Tellier and Brown 2007) to multiple loci. Polycyclic disease generates direct FDS for parasite virulence \( \partial \Delta f_{aa} / \partial f_{aa} < 0 \) and \( \partial \Delta f_{aa} / \partial f_{aa} < 0 \) but not for host resistance \( \partial \Delta f_{rr} / \partial f_{rr} = \partial \Delta f_{rr} / \partial f_{rr} = 0 \). Increasing allo-infection (decreasing \( \psi \)) tends to make successive parasite generations on the same plant independent of one another, causing selection against parasite genotypes to tend to become independent of their own frequency (Tellier and Brown 2007), and decreasing the parameter space in which polymorphism is stable. This can be explained as follows.

The coefficient \( \partial \Delta f_{aa} / \partial f_{aa} \) tends to zero when \( \psi \) is small. When the frequency \( (AA) \) of the double-AVR parasite (genotype \( II \)) is high and \( \psi \) is low, most double-RES plants infected by double-avr parasites in \( (g,1) \) then encounter a double-AVR parasite in \( (g,2) \). Increasing \( \psi \), however, increases the probability of these double-RES plants remaining infected with an double-avr parasite in \( (g,2) \). Hence at higher frequencies of double-AVR parasites and increasing auto-infection, the strength of natural selection for the double-avr genotype and against double-AVR becomes greater \( \partial \Delta f_{aa} / \partial f_{aa} \) is more negative). Similarly, the coefficient \( \partial \Delta f_{aa} / \partial f_{aa} \) tends to zero when \( \psi \) is low. When the frequency \( (Aa) \) of parasite
genotype 10 and ψ are both low, most 01 plants infected by 10 parasites in (g,1) then encounter a 01 parasite in (g,2). Increasing ψ, however, increases the probability of these 01 plants remaining infected with an 10 parasite in (g,2). Hence, natural selection for parasite genotype 10 and against 01 is stronger (\( \partial \Delta f_{a} / \partial f_{a} \) is more negative) when the frequency of 10 parasites is lower, and this effect is stronger as ψ increases.

The absence (Model B1, Figures 1-2) or presence (Model B2, Figures 3-4) of epistatic interactions between fitness costs at different GFG loci have a considerable influence on the maintenance of multiple genotypes in host and parasite populations. Epistatic interactions between virulent loci allow the existence of stable equilibrium frequencies of plants genotypes with multiple RES alleles. In two locus systems, double resistant plants (11) are maintained if \( avr \) alleles have a negative synergistic effect on parasite fitness (Model B2), and these results extend to three locus interactions (Model C in ESM, section 7). In biological terms, increasing exponentially the cost of each \( avr \) allele as a function of the total number of \( avr \) alleles counter-selects genotypes with multiple \( avr \) alleles. The resulting higher parasite genotypic diversity then favours multiple-RES plant genotypes. Moreover, when the cost of each \( RES \) allele diminishes as a function of the total number of \( RES \) alleles, this counter-selects host genotypes with intermediate numbers of \( RES \) alleles, and favours double-res plants. Parasites with few \( AVR \) alleles are then favoured against super-\( avr \) parasites which have high costs of \( avr \). As a consequence, the equilibrium point with all four parasite genotypes is more likely to exist.

Epistatic interactions between \( RES \) and \( avr \) loci are interesting in relation to current advances in research on the function of \( RES \) and \( avr \) genes. To date, the great majority of experiments on plant and parasite fitness have been conducted on single genes (\( RES \) or \( avr \)). Some (Leonard 1969; Thrall and Burdon 2003; Tian et al. 2003; Vera Cruz et al. 2000) but not all (Bergelson and Purrington 1996; Brown 2003; Vera Cruz et al.
2000) experiments have detected such costs. The structure of fitness costs emerging from current research in molecular biology supports the hypothesis that epistasis is of the type that leads to GFG polymorphism being stable (and hence detectable).

Avirulence genes in plant parasites have a dual role. The proteins they encode are recognised by the host plant’s defence machinery, hence their avirulence function, similar to the antigenicity of parasites of vertebrates. However, many AVR proteins also have effector activity, promoting infection, colonisation or pathogenicity (Jones and Dangl 2006; Ridout et al. 2006; Skamnioti and Ridout 2005). Parasites generally have numerous avirulence/effector genes (Kay et al. 2005) and there is evidence for redundancy between AVR proteins (Bai et al. 2000; Kay et al. 2005; Mudgett 2005; Skamnioti and Ridout 2005; Wichmann and Bergelson 2004). Increasing the number of mutations of AVR genes to avr alleles may therefore have a synergistic negative effect on parasite fitness (infectivity, growth, reproduction, etc). In experiments on multiple knockouts of avirulence/effector genes in Xanthomonas axonopodis, the loss of function of one or two of four avirulence genes did not affect significantly bacterial growth, but a significant effect was observed when three or four genes were knocked out (Wichmann and Bergelson 2004). Several parasite AVR genes exist as gene families and are thus predicted to complement each other’s effector function to some extent (e.g. Blumeria graminis, Ridout et al. 2006; Skamnioti and Ridout 2005). Whether such a situation is general is not yet known, but if the cost of having one or very few avr alleles is low (2% in model B2), it might explain the lack of experimental evidence for a high cost of a single virulence allele (Thrall and Burdon 2003; Vera Cruz et al. 2000). It is also predicted that polymorphism would be commonly observed in multi-gene families where there is synergistic epistasis of the costs of different avr alleles, as in Models B2 and C. An experiment to test this prediction would estimate the fitness costs of combinations of
various numbers of \textit{AVR} genes in a common background (\textsc{Wichmann} and \textsc{Bergelson 2004}).

Antagonistic interactions between \textit{RES} alleles in their effect on host fitness are not essential to maintain polymorphism in parasite genotypes, but favour maintenance of genotypes with a low to intermediate number of \textit{avr} alleles. We assume here that the cost of having one \textit{RES} allele is high (7\% in our simulations compared to 9\% found by Tian et al. 2003), but that the marginal cost of each new \textit{RES} allele added at other loci diminishes (here only 2\% more cost for the second allele). High costs of \textit{RES} have been found experimentally (Tian et al. 2003), but if different \textit{RES} genes each had such a cost, the fitness of a plant with several \textit{RES} genes would be severely depressed (Brown 2003). However, if the marginal cost of adding a new \textit{RES} gene is small, the fitness load of many \textit{RES} genes may be little greater than that of one (Bergelson and Purrington 1996; Palomino et al. 2002). Functional data on resistance reactions show that many \textit{RES} genes activate similar defence proteins (Jones and Dangl 2006). The cost of expressing host defences may be similar whether they are triggered by a single \textit{RES-AVR} interaction or by several pairs of \textit{RES} and \textit{AVR} genes.

Other theoretical models have suggested that multi-locus GFG systems for monocyclic disease enhance polymorphism maintenance (Frank 1993a; Frank 1997; Salathe et al. 2005; Sasaki 2000; Segarra 2005; Thrall and Burdon 2002). In a multi-locus GFG co-evolutionary system with \( n \) interacting loci, there are \( 2^n \) genotypes in host and parasite. An increase in \( n \) also diminishes the expected frequency of each host and parasite genotype to a mean equilibrium frequency of \( 1/2^n \) (Frank 1993a; Frank 1997). In finite and spatially structured populations, allele frequencies in a high dimension system (high \( n \)) are thus more sensitive to random processes (mutations, genetic drift, and migrations) counteracting the frequency-dependent selection process (Frank 1993a; Frank 1997;
Thrall and Burdon 2002). High mutation rates (Salathe et al. 2005; Sasaki 2000; Segarra 2005) introduce new genotypes at high frequencies and sustain successive stochastic frequency-dependent selection cycles. As there is no direct frequency-dependent selection in simple models of monocyclic disease, mutation lead to arms race co-evolutionary dynamics, with recurrent fixation of alleles, rather than trench warfare dynamics, with stable polymorphism. Interestingly, in our models, realistic rates of mutation do not affect the outcome of co-evolution in terms of the stability of polymorphism or the number of genotypes maintained but merely increase the time to genotype fixation and the life time of a mutation (this was also shown by Segarra 2005).

Polycyclic disease and auto-infection are important features of many diseases of plants and animals and have been shown to favour stable long term maintenance of polymorphism at host and parasite loci (Tellier and Brown 2007). Here, a similar outcome is observed in multi-locus GFG interactions with realistic costs of RES and avr alleles (Model B1), in contrast to monocyclic disease (Model A). Models B2 and C indicate the importance of epistatic interactions between host and parasite loci for costs of multiple RES and avr alleles and predict that GFG polymorphism will be stable (and hence detectable) when there is precisely the structure of costs that seems to be emerging from current discoveries in molecular biology.
**LITERATURE CITED**


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APPENDIX

Two locus GFG model with monocyclic disease

The model: The parasite genotype frequencies at host generation \( g \) are \( AA_g, Aa_g, aA_g \) and \( aa_g \) for the genotypes 11, 10, 01 and 00 respectively. Similarly, \( rr_g, rR_g, Rr_g \) and \( RR_g \) stand for the frequencies in generation \( g \) of the respective host genotypes 11, 10, 01 and 00.

The frequency of parasite genotype 11 in host generation \( g+1 \) is thus: \( AA_{g+1} = AA_g rR_g/w_A \)

Similarly,

\[
\begin{align*}
aa_{g+1} &= aa_g(1-b_2)/w_A \\
aA_{g+1} &= aA_g(1-b_1)(rR_g + Rr_g)/w_A \text{ and} \\
Aa_{g+1} &= Aa_g(1-b_1)(rr_g + rR_g)/w_A \\
\end{align*}
\]

Where \( w_A \) is the overall parasite population fitness:

\[
\begin{align*}
\overline{w_A} &= AA_g rr_g + aa_g(1-b_2) + aA_g(1-b_1)(rr_g + Rr_g) + Aa_g(1-b_1)(rr_g + rR_g) \\
\end{align*}
\]

Host 11 genotype frequency at host generation \( g+1 \) is then:

\[
\begin{align*}
RR_{g+1} &= RR_g(1-u_a)(aa_g(1-s) + AA_g + AA_g)/w_R \text{ and} \\
Rr_{g+1} &= Rr_g(1-u_a)((1-s)(aa_g + aA_g) + Aa_g + AA_g)/w_R \\
rR_{g+1} &= rR_g(1-u_a)((1-s)(aa_g + Aa_g) + aA_g + AA_g)/w_R \\
rR_{g+1} &= rR_g(1-s)/w_R \\
\end{align*}
\]

Where \( w_R \) is the overall host population fitness:

\[
\begin{align*}
\overline{w_R} &= \begin{bmatrix}
RR_g(1-u_a)(aa_g(1-s) + AA_g + AA_g) + Rr_g(1-u_a)((1-s)(aa_g + aA_g) + Aa_g + AA_g) \\
+ rR_g(1-u_a)((1-s)(aa_g + Aa_g) + aA_g + AA_g) + rr_g(1-s)
\end{bmatrix}
\end{align*}
\]
**Stability of the equilibrium state:** Using logit transformations of the above equations (Eqs. [5,6] in text), the Jacobian matrix $J_A$ can be rewritten:

$$
J_A = 
\begin{pmatrix}
\frac{\partial \Delta f_{aa}}{\partial f_{aa}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} \\
\frac{\partial \Delta f_{aa}}{\partial f_{aa}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} \\
\frac{\partial \Delta f_{aa}}{\partial f_{aa}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} \\
\frac{\partial \Delta f_{aa}}{\partial f_{aa}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} & \frac{\partial \Delta f_{aa}}{\partial f_{rr}} \\
\end{pmatrix}
$$

The coefficients of $J_A$ are the rates of natural selection of the ratio of genotype frequencies. For example, $\frac{\partial \Delta f_{aa}}{\partial f_{rr}}$ is the rate of selection on the double-avr parasite genotype (00) as a function of the frequency of the double-susceptible host genotype (00). Close to the equilibrium point, the Jacobian matrix coefficients are approximately (ESM, Section 1):

$$
J_A = 
\begin{pmatrix}
0 & 0 & \frac{h^2(1-h_1)^2}{1-h_1} & 0 \\
0 & 0 & 0 & \frac{2b_1 - b_2 - 2bh_2}{1-h_1} \\
-\frac{(1-s)(u_2-u_1)^2}{s(1-u_1)} & 0 & 0 & 0 \\
0 & \frac{(s-u_2)(2u_1-u_2-u_1)}{s(1-u_1)(1-u_2)} & 0 & 0 \\
\end{pmatrix}
$$

The Jacobian matrix $J_A$ is diagonalizable, and thus has four eigenvalues ($\lambda_{1,4}$):

$$
\begin{cases}
\lambda_{1,2} = \pm \sqrt{-\beta_1} & \text{if } \beta_1 < 0 \\
\lambda_{3,4} = \pm \sqrt{\beta_2} & \text{if } \beta_1 > 0
\end{cases}
$$

with $\beta_1 = \frac{(b_1 - 2h_1(1-h_2))(u_2-u_1)(2-u_2)(s-u_2)}{s(1-h_1)(1-u_1)(1-u_2)}$ and $\beta_2 = \frac{(1-s)(1-h_2)b_2^2(u_1-u_2)^2}{s(1-h_1)(1-u_2)}$

The sign of $\beta_1$ depends on values of costs ($u_1, u_2, b_1, b_2, s$), and $\beta_2$ is always always positive.
Table 1: Host and parasite fitnesses for monocyclic disease and two loci in each species interacting by gene-for-gene relationships.

| Parasite fitness | Host fitness | | | | | | |
|------------------|--------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | AA | Aa | aA | aa | AA | Aa | aA | aa |
| RR               | 0  | 0  | 0  | 1-b2 | 1-u2 | 1-u2 | 1-u2 | (1-s)(1-u2) |
| Rr               | 0  | 0  | 1-b1 | 1-b2 | 1-u1 | 1-u1 | (1-s)(1-u1) | (1-s)(1-u1) |
| rR               | 0  | 1-b1 | 0  | 1-b2 | 1-u1 | (1-s)(1-u1) | 1-u1 | (1-s)(1-u1) |
| rr               | 1  | 1-b1 | 1-b1 | 1-b2 | 1-s  | 1-s  | 1-s  | 1-s  |
Table 2: Fitness of hosts and parasites in Model B for interactions with two parasite generations per host generation ($G=2$) and only auto-infection between parasite generations ($\psi=1$).

<table>
<thead>
<tr>
<th>Parasite genotypes (frequencies) within host generation $g$</th>
<th>Fitness at beginning of host generation $g+1$</th>
<th>Fitness of 2nd parasite infection</th>
<th>Host fitness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st generation</td>
<td>2nd generation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 ($AA_g$)</td>
<td>11 ($AA_{g+1}$)</td>
<td>0</td>
<td>1-$u_{2}$</td>
</tr>
<tr>
<td>10 ($Aa_g$)</td>
<td>10 ($Aa_{g+1}$)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>01 ($aA_g$)</td>
<td>01 ($aA_{g+1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 ($RR_g$)</td>
<td>00 ($aa_{g+1}$)</td>
<td>1-$b_{2}$</td>
<td>(1- $u_{2}$)(1-$c$)</td>
</tr>
<tr>
<td>10 ($Aa_g$)</td>
<td>10 ($Aa_{g+1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00 ($aa_g$)</td>
<td>00 ($aa_{g+1}$)</td>
<td>1-$b_{2}$</td>
<td>(1- $u_{2}$)(1-$\phi$)</td>
</tr>
<tr>
<td>00 ($aa_g$)</td>
<td>00 ($aa_{g+1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Host genotype 01 ($Aa_g$)</td>
<td>10 ($Aa_{g+1}$)</td>
<td>1-$b_{1}$</td>
<td>(1-$\phi$)</td>
</tr>
<tr>
<td>10 ($Aa_g$)</td>
<td>10 ($Aa_{g+1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00 ($aa_g$)</td>
<td>00 ($aa_{g+1}$)</td>
<td>1-$b_{2}$</td>
<td>(1- $u_{1}$)(1-$\phi$)</td>
</tr>
<tr>
<td>00 ($aa_g$)</td>
<td>00 ($aa_{g+1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Host genotype 11 ($AA_g$)</td>
<td>11 ($AA_{g+1}$)</td>
<td>0</td>
<td>1-$u_{1}$</td>
</tr>
<tr>
<td>10 ($Aa_g$)</td>
<td>10 ($Aa_{g+1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>01 ($aA_g$)</td>
<td>01 ($aA_{g+1}$)</td>
<td>1-$b_{1}$</td>
<td>(1- $u_{1}$)(1-$c$)</td>
</tr>
<tr>
<td>10 ($Aa_g$)</td>
<td>10 ($Aa_{g+1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00 ($aa_g$)</td>
<td>00 ($aa_{g+1}$)</td>
<td>1-$b_{2}$</td>
<td>(1- $u_{1}$)(1-$\phi$)</td>
</tr>
<tr>
<td>00 ($aa_g$)</td>
<td>00 ($aa_{g+1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Host genotype 10 ($RR_g$)</td>
<td>11 ($AA_{g+1}$)</td>
<td>0</td>
<td>1-$u_{1}$</td>
</tr>
<tr>
<td>11 ($AA_g$)</td>
<td>11 ($AA_{g+1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 ($Aa_g$)</td>
<td>10 ($Aa_{g+1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>01 ($aA_g$)</td>
<td>01 ($aA_{g+1}$)</td>
<td>1-$b_{1}$</td>
<td>(1- $u_{1}$)(1-$c$)</td>
</tr>
<tr>
<td>01 ($aA_g$)</td>
<td>10 ($Aa_{g+1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>00 ($aa_g$)</td>
<td>00 ($aa_{g+1}$)</td>
<td>1-$b_{2}$</td>
<td>(1- $u_{1}$)(1-$\phi$)</td>
</tr>
<tr>
<td>01 ($aA_g$)</td>
<td>10 ($Aa_{g+1}$)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIGURES

Figure 1: Stability area plots for a two-locus model with independent costs of alleles at different loci (Model B1), in relation to values of the autoinfection rate ($\psi$) and the cost to a plant of being diseased by two parasite generations ($\phi$). $u_1$, $u_2$: costs of host resistance; $b_1$, $b_2$: costs of parasite virulence; $u_1=b_1= 0.05$, $u_2=b_2= 0.0975$ (subscript 1: cost of one allele; subscript 2: cost of two alleles).

(a) Stability of polymorphism in the host population. Area A: Fixation of double-susceptible genotype 00; Area B: Stable polymorphism with 00 and the single-resistant genotypes 10 and 01; Area D: Unstable polymorphism with 00, 10, 01; Area E: Fixation of 00.

(b) Stability of polymorphism in the parasite population. Area G: Fixation of the double-avirulent genotype 11; Area H: Stable polymorphism with 11 and the single-avirulent genotypes 01 and 10; Area I: Stable polymorphism with all four genotypes; Area J: Stable polymorphism with 10, 01 and the double-virulent genotype 00; Area K: Unstable polymorphism with 10, 01, 00; Area L: Fixation of 00.

Figure 2: Dynamics of host and parasite genotype frequencies in a two-locus model with independent costs of alleles at different loci (Model B1) defined by two loci as a function of the number of host generations. There is no mutation. Auto-infection rate $\psi=0.9$ and maximum cost of disease $\phi=0.2$. $u_1$, $u_2$: costs of host resistance; $b_1$, $b_2$: costs of parasite virulence; $u_1=b_1= 0.05$, $u_2=b_2= 0.097$. (a) Maintenance of host genotypes with one or both susceptibility alleles but the double-resistant genotype 11 is eliminated (Area B in Figure 1a). (b) Maintenance of all four parasite genotypes (Area I in Figure 1b).
Figure 3: Stability area plots for a two-locus model with epistasis in costs of alleles at different loci (Model B2), in relation to values of the autoinfection rate ($\psi$) and the cost to a plant of being diseased by two parasite generations ($\phi$). $u_1, u_2$: costs of host resistance; $b_1, b_2$: costs of parasite virulence; $u_1=0.072$ and $b_1=0.024$; $u_2=b_2=0.0975$.

(a) Stability of polymorphism in the host population. Area A: Fixation of double-susceptible genotype 00; Area B: Stable polymorphism with 00 and the single-resistant genotypes 10 and 01; Area C: Stable polymorphism with all four genotypes (10, 01, 00, 11); Area F: Unstable polymorphism with all four genotypes; Area E: Fixation of 00.

(b) Stability of polymorphism in the parasite population. Area G: Fixation of the double-avirulent genotype 11; Area H: Stable polymorphism with 11 and the single-avirulent genotypes 01 and 10; Area I: Stable polymorphism with all four genotypes (10, 01, 00, 11); Area M: Unstable polymorphism with all four genotypes; Area L: Fixation of the double-virulent genotype 00.

Figure 4: Dynamics of host and parasite genotype frequencies in a two-locus model with epistasis in costs of alleles at different loci (Model B2) as a function of the number of host generations. There is no mutation. Auto-infection rate $\psi=0.9$ and maximum cost of disease $\phi=0.2$. $u_1, u_2$: costs of host resistance; $b_1, b_2$: costs of parasite virulence; $u_1=0.072$ and $b_1=0.024$; $u_2=b_2=0.0975$. (a) Maintenance of all four host genotypes (Area C in Figure 3a). (b) Maintenance of all four parasite genotypes (Area I in Figure 3b).
Figure 1A

Figure 1B
Figure 2A

- Host genotype frequencies

Figure 2B

- Parasite genotype frequencies
Figure 4A

Figure 4A shows the host genotype frequencies over host generations. The x-axis represents the host generations ranging from 1000 to 3000, while the y-axis represents the genotype frequencies ranging from 0.0 to 1.0. The graph includes four genotypes: 00, 10, 01, and 11, each represented by a different line.

Figure 4B

Figure 4B illustrates the parasite genotype frequencies over host generations. The x-axis is the same as in Figure 4A, representing host generations from 1000 to 3000. The y-axis shows the parasite genotype frequencies from 0.0 to 1.0. The graph includes four genotypes: 00, 10, 01, and 11, each marked with a unique line style.